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(54) **PENICILLIUM AMAGASAKIENSE GLUCOSE OXIDASE MUTANTS**

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**C07K 1/00** (2006.01)

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C12Q 1/006  
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435/91.1, 320.1, 252.3; 536/23.1, 23.2;  
530/350

See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to mutants of the *Penicillium amagasakiense* glucose oxidase (GOx) enzyme which are of use for assaying glucose and to the development in particular of glucose electrodes and of biocells which use glucose as fuel.

**16 Claims, 4 Drawing Sheets**

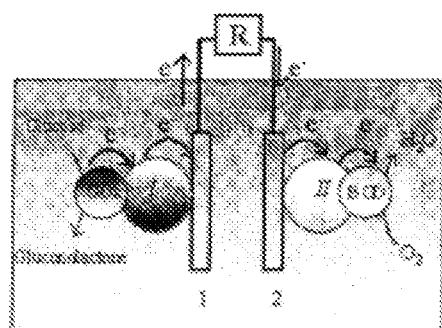


Figure 1

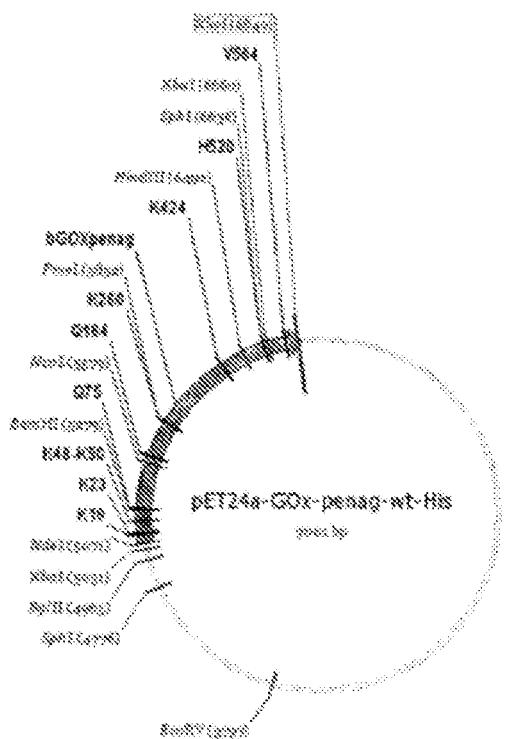


Figure 2

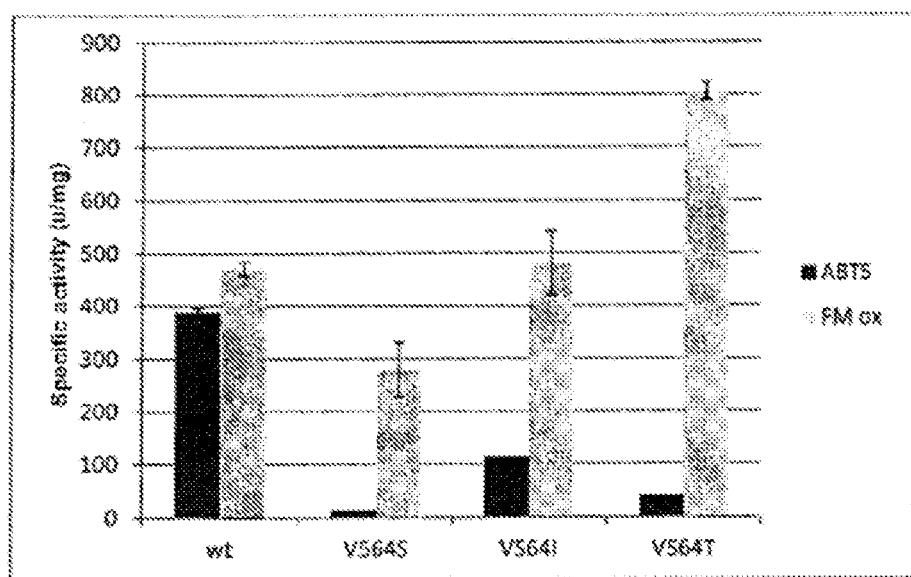


Figure 3

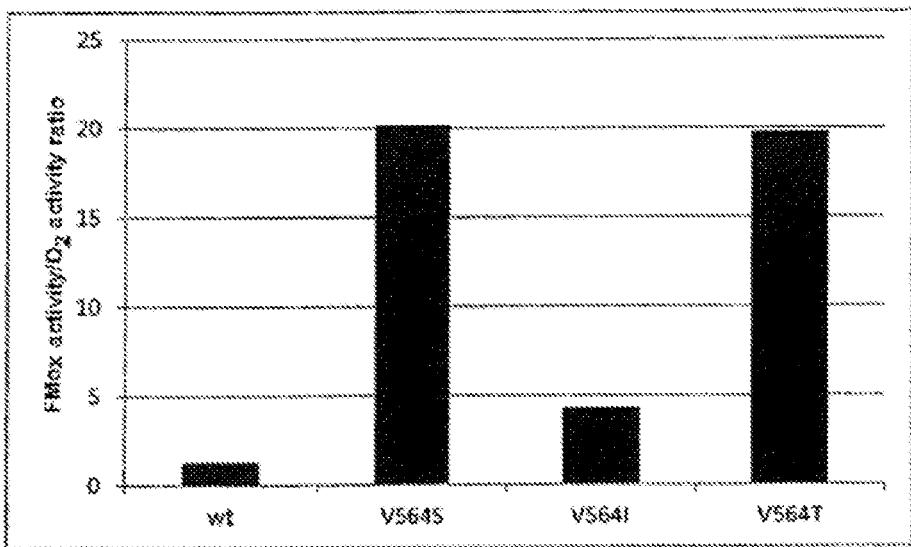


Figure 4

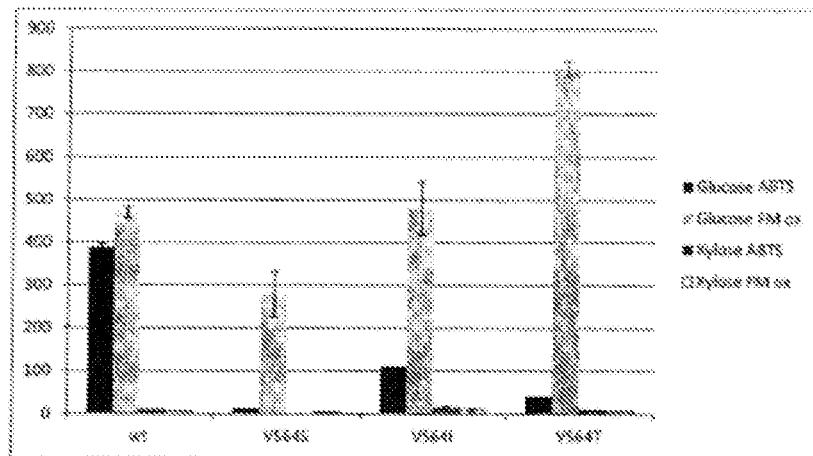


Figure 5

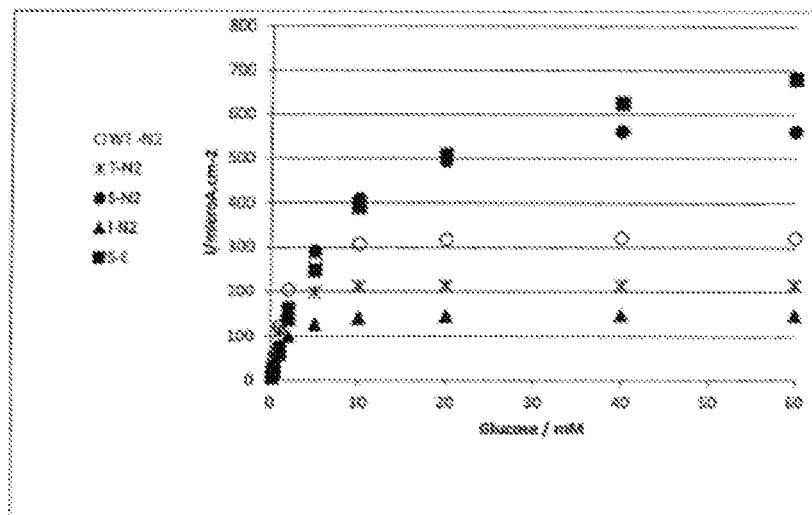


Figure 6

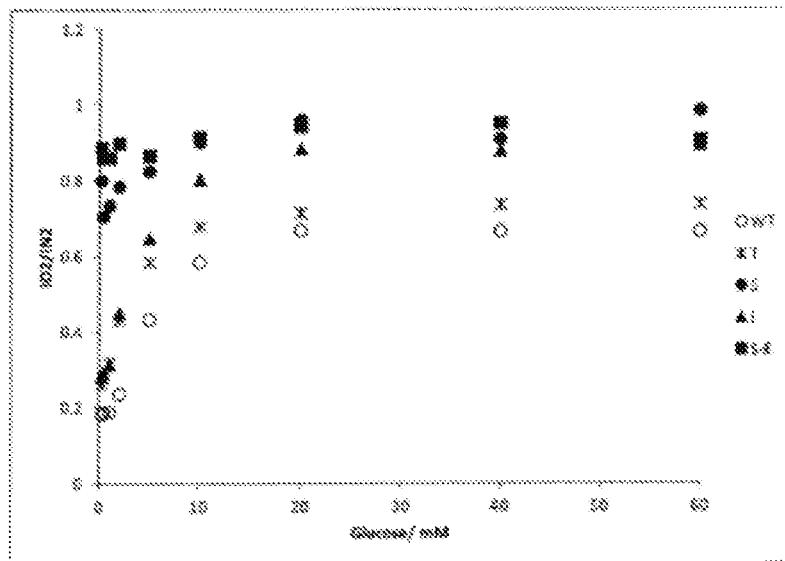


Figure 7

## 1

**PENICILLIUM AMAGASAKIENSE GLUCOSE OXIDASE MUTANTS**

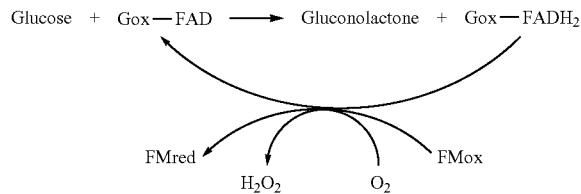
The present invention relates to the field of developing glucose electrodes which are of interest in assaying glucose, in particular the blood glucose of diabetic individuals, and for the use of biocells using glucose as fuel.

The present invention is more particularly directed toward mutants of the glucose oxidase enzyme (also referred to hereinbelow as GOx) of *Penicillium amagasakiense* which have advantageous properties over the wild-type enzymes, in particular the commercialized enzymes.

Type-2 diabetes affects nearly two million people in France, added to which are 600 000 people who are unaware of their disease. In the United States, the situation is even more critical. In developed countries, diabetes is the main cause of blindness among 20-65 year olds.

The monitoring and surveillance of the disease is based, inter alia, on daily assay of the blood glucose and the injection of insulin. Various companies propose glucose sensors that enable patients to measure their glycemia at home. These sensors may be amperometric, potentiometric or coulometric; they are all based on the use of an enzyme that is capable of oxidizing glucose; the two main enzymes being glucose oxidase and PQQ s-GDH.

Glucose oxidase (also referred to hereinbelow as GOx) is an oxidoreductase enzyme (EC 1.1.3.4) which catalyzes the oxidation of glucose to hydrogen peroxide and D-glucono- $\delta$ -lactone according to the following reaction scheme:



Glucose oxidase was isolated for the first time from *Aspergillus niger*; the GOx most conventionally produced are those from *Penicillium chrysogenum*, *Penicillium glaucum*, *Penicillium purpurogenum*, *Penicillium amagasakiense*, *Aspergillus niger* and *Aspergillus fumigatus*.

The marketed GOx are usually those from *Aspergillus niger* and *Penicillium amagasakiense*; they are mainly used in the food industry, especially for conservation purposes as a source of hydrogen peroxide. They are also used for assaying glucose or in glucose biocells. These two enzymes were especially studied and compared in the article by Wohlfahrt et al. (Acta. Cryst (1999) D55, 969-977).

As regards GOx from *Penicillium amagasakiense*, Witt et al. described its cloning and its expression with *Escherichia coli* (Applied and Environmental Microbiology (1998) vol. 64, No. 4, 1405-1411).

The drawback of the currently available glucose oxidases is their sensitivity to O<sub>2</sub> which participates as an electron acceptor in the reaction catalyzed by GOx. Specifically, oxygen is the natural cofactor of GOx and enables their reoxidation after the oxidation of glucose. During the use of these enzymes in glucose sensors, there is thus competition for recovery of the electrons from the enzyme between oxygen and the redox mediators which provide the electrical connection of the enzyme to the surface of the electrodes.

In addition, for their use in glucose sensors, it is necessary to have available more active GOx mutants, i.e. mutants

## 2

which allow a faster transformation reaction of glucose to D-gluconolactone than with the existing enzymes.

It thus remains necessary to improve the properties of the existing GOx.

5 This is what the Inventors have managed to do by developing novel mutants of the wild-type GOx of *Penicillium amagasakiense*.

The term "mutant or variant" means a GOx whose protein sequence comprises the insertion, deletion and/or replacement of at least one amino acid relative to the protein sequence of the wild-type GOx; hereinbelow, the reference nucleotide and protein sequences of GOx are those of the wild-type GOx of *Penicillium amagasakiense* (respectively SEQ. ID. No. 1 and 2).

10 The mutants according to the present invention are such that the valine in position 564 is replaced with a serine (V564S mutant), a threonine (V564T mutant) or an isoleucine (V564I mutant); when said valine is replaced with a serine, 15 the mutants such that the lysine in position 424 is replaced with a glutamic acid, glutamine, methionine and leucine are also subjects of the present invention (V564S+K424E, V564S+K424Q, V564S+K424M and V564S+K424L mutants, respectively).

20 Thus, a first subject of the invention relates to a GOx mutant with a percentage of identity of at least 80%, and, in order of increasing preference, at least 85%, 90%, 95%, 97%, 25 98% and 99%, relative to the wild-type GOx of *Penicillium amagasakiense*, characterized in that its amino acid in position 564, with reference to the protein sequence of the wild-type GOx of *Penicillium amagasakiense* (SEQ. ID. No. 2), is replaced with an amino acid selected from the group consisting of a serine (V564S mutant), a threonine (V564T mutant) or an isoleucine (V564I mutant).

30 According to a particular variant, the V564S mutant also comprises a replacement of the lysine in position 424 with a glutamic acid, glutamine, methionine or leucine (V564S+K424E, V564S+K424Q, V564S+K424M and V564S+K424L mutants, respectively).

35 40 The numbering of the amino acids refers to the sequence of the wild-type GOx of *Penicillium amagasakiense*.

The identity of a sequence relative to the sequence of the wild-type GOx of *Penicillium amagasakiense* (SEQ. ID. No. 2) as reference sequence is assessed as a function of the 45 percentage of amino acid residues that are identical, when the two sequences are aligned, so as to obtain the maximum correspondence between them.

The percentage of identity may be calculated by a person skilled in the art using a computer program for comparing 50 sequences, for instance the BLAST software (Altschul et al., NAR, 25, 3389-3402). The BLAST programs are used on the comparison window consisting of all of the SEQ. ID. No. 2 indicated as the reference sequence.

A peptide with an amino acid sequence having at least X % 55 identity with a reference sequence is defined in the present invention as a peptide whose sequence may include up to 100-X alterations per 100 amino acids of the reference sequence, while conserving the functional properties of said reference peptide, in the present case its enzymatic activity 60 for the oxidation of glucose. For the purposes of the present invention, the term "alteration" includes consecutive or dispersed deletions, replacements or insertions of amino acids in the reference sequence.

The amino acid corresponding to the amino acid in position 65 564 of the wild-type GOx of *Penicillium amagasakiense* is identified by aligning the sequence of said homologous enzyme with the GOx of *Penicillium amagasakiense*.

A particular subject of the invention relates to a GOx mutant with an amino acid sequence chosen from SEQ. ID. No. 4, 6, 8, 10, 22, 24 and 26 corresponding, respectively, to the amino acid sequences of the mutants V564S, V564T, V564I, V564+K424E, V564S+K424Q, V564S+KL424M and V564S+K424L of GOx; these mutated enzymes are encoded by nucleotide fragments obtained by mutation of the wild-type GOx gene of *Penicillium amagasakiense* with adapted pairs of oligonucleotides.

These novel GOx mutants according to the invention have improved performance qualities over the wild-type enzyme of *Aspergillus niger* which is the enzyme used in commercial glucose sensors.

More particularly, the improved properties of the mutants according to the invention lie in a reduced sensitivity to oxygen: in solution in the presence of 1 mM of glucose and in air, the mutants are 17 times less sensitive to oxygen than the GOx of *A. niger*. Once adsorbed onto the surface of electrodes, under 1 atm of O<sub>2</sub> and at 1 mM of glucose, the mutants are 70% less sensitive to O<sub>2</sub>.

The advantageous properties of the GOx mutants according to the invention make their use particularly suited to bioelectric systems such as biocells using glucose as a source of energy and glucose biosensors.

The present invention also relates to a nucleic acid molecule coding for a GOx mutant according to the invention; said nucleic acid molecule being obtained by modification of a wild-type GOx, such as that of *Penicillium amagasakiense*, with an oligonucleotide pair selected from the group consisting of the oligonucleotide pairs represented in Table I.

TABLE I

sequence listing of the oligonucleotides used for the preparation of the GOx mutants according to the invention	
Oligo-nucleotides	Sequences
Oligonucleotide pair corresponding to the wild-type enzyme	
Sense	5'-g gtg tct tcc cat gtc atg acc att ttc tac gg-3' (SEQ. ID. No. 11)
Antisense	5'-cc gta gaa aat ggt cat gac atg gga aga cac c-3' (SEQ. ID. No. 12)
Oligonucleotide pair used for the preparation of the V564S mutant	
Sense	5'-g gtg tct tcc cat tcc atg acc att ttc tac gg-3' (SEQ. ID. No. 13)
Antisense	5'-cc gta gaa aat ggt cat gga atg gga aga cac c-3' (SEQ. ID. No. 14)
Oligonucleotide pair used for the preparation of the V564T mutant	
Sense	5'-g gtg tct tcc cat acc atg acc att ttc tac gg-3' (SEQ. ID. NO. 15)
Antisense	5'-cc gta gaa aat ggt cat ggt atg gga aga cac c-3' (SEQ. ID. No. 16)

TABLE I-continued

sequence listing of the oligonucleotides used for the preparation of the GOx mutants according to the invention	
Oligo-nucleotides	Sequences
Oligonucleotide pair used for the preparation of the V564I mutant	
Sense	5'-g gtg tct tcc cat att atg acc att ttc tac gg-3' (SEQ. ID. NO. 17)
Antisense	5'-cc gta gaa aat ggt cat aat atg gga aga cac c-3' (SEQ. ID. No. 18)
Oligonucleotide pair used for the preparation of the K424E mutation of the V564S-K424E mutant (this mutation is performed after the V564S mutation)	
Sense	5'-ggacaccgaggggcagatcaacttcg-3' (SEQ. ID. No. 19)
Antisense	5'-cgaagtgtatcgccctcggtgtcc-3' (SEQ. ID. No. 20)
Oligonucleotide pair used for the preparation of the K424Q mutation of the V564S-K424Q mutant (this mutation is performed after the V564S mutation)	
Sense	5'-ggacaccgaggggcagatcaacttcgat ttatg-3' (SEQ. ID. No. 27)
Antisense	5'-cataaatcgaagtgtatctggccctcg tgtcc-3' (SEQ. ID. No. 28)
Oligonucleotide pair used for the preparation of the K424M mutation of the V564S-K424M mutant (this mutation is performed after the V564S mutation)	
Sense	5'-ggacaccgaggggcatgtatcaacttcgat ttatg-3' (SEQ. ID. No. 29)
Antisense	5'-cataaatcgaagtgtatcatggccctcg tgtcc-3' (SEQ. ID. No. 30)
Oligonucleotide pair used for the preparation of the K424L mutation of the V564S-K424L mutant (this mutation is performed after the V564S mutation)	
Sense	5'-ggacaccgagggggttgcacttcgat ttatg-3' (SEQ. ID. No. 31)
Antisense	5'-cataaatcgaagtgtatcaaggccctcg tgtcc-3' (SEQ. ID. No. 32)

The nucleic acid molecules coding for the GOx mutants according to the invention may especially be prepared by modifying the nucleotide sequence of the gene coding for the wild-type enzyme of sequence SEQ. ID. No. 1 produced by *Penicillium amagasakiense*. Several techniques for modifying the gene sequence are known to those skilled in the art (see the review by Igarashi et al., Archives of Biochemistry and Biophysics 428 (2004) 52-63). In a particular mode of preparation, the nucleic acid molecules coding for the GOx mutants according to the invention are prepared by mutagenesis by PCR in the presence of an oligonucleotide bearing the mutation to be introduced (see the experimental section, point 2.5 below).

According to a particular embodiment, the present invention relates to a nucleic acid molecule coding for a GOx mutant according to the invention whose sequence is selected from the group consisting of sequences SEQ. ID. No. 3, 5, 7, 9, 21, 23 and 25. The nucleic acid molecules coding for the GOx mutants according to the invention may then be cloned in an expression vector such as a plasmid, and then transformed in a suitable host such as a bacterium, a yeast or a cell culture.

The term "expression vector" means a vector bearing a region for insertion of a coding nucleotide sequence between the signals that are essential for its expression, especially a promoter (constitutive or inducible), a ribosome binding site, a transcription termination signal, and, optionally, a selection marker such as a gene for resistance to an antibiotic.

The present invention also relates to an expression vector comprising said nucleic acid molecule and to a host cell transformed with said expression vector and expressing a GOx mutant according to the invention.

The introduction of the expression vector into the host cell may be performed via any method known to those skilled in the art, in particular by modification of the membrane permeability of the host cell, for example in the presence of calcium ions, or by electroporation.

After culturing the transformed host cells to express a GOx mutant according to the invention, said cells may be recovered by centrifugation, lysed so as to release the enzymes including said GOx mutant according to the invention.

If *Escherichia coli* is the host microorganism, the plasmids that may be used are especially the plasmids pET24a, pBlue-script, pUC18 or the like.

By way of example, the host cells that may be used comprise *Escherichia coli* BL<sub>21</sub>, *Escherichia coli* W3110, *Escherichia coli* C600, *Escherichia coli* JM109, *Escherichia coli* JM101, *Escherichia coli* DH5α, etc.

Preferably, the GOx mutants according to the invention are produced in a strain of *Escherichia coli* BL<sub>21</sub>; the nucleic acid molecule which codes them is obtained by modification of the GOx gene of *Penicillium amagasakiense* and cloned in the vector pET24a. The mutants thus produced are exported into the periplasm of the bacterium by means of the signal sequence of GOx. The mutants thus produced are exported in the inclusion bodies of the bacterium. The mutants are then purified after rupturing the bacteria by cell lysis in the presence of 8M urea.

The invention also relates to the use of a GOx mutant according to the invention for assaying glucose in solution, i.e. for measuring the concentration of glucose in a sample, especially a biological sample, in particular in blood.

The assay of glucose in solution in a given biological sample may be performed by introducing into said sample a redox reagent and a GOx mutant according to the invention and then by comparing the intensity of the coloration obtained with standard solutions having a known glucose content.

The present invention also relates to a kit for assaying a glucose solution, characterized in that it comprises a GOx mutant according to the invention.

Typically, said assay kit also contains the reagents necessary for performing the glucose assay test, in particular buffers; any buffer may be used in the kit according to the invention, mention may be made without any limiting nature of phosphate or acetate buffers, tris(hydroxymethyl)aminomethane (TRIS) buffer, N-morpholino-3-propanesulfonic acid (MPOS), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), buffer comprising a mixture of buffers such as TRIS-acetate, etc., the redox reagents may be any

reagent that allows the GOx mutant to be oxidized, and may be selected from the group consisting of phenazine methosulfate (PMS) in combination with 2,6-dichlorophenolindophenol (DCIP); potassium ferricyanide; ferrocene and ferrocene-based complexes such as ferrocenemethanol, ferrocenecarboxylic acid; and osmium and ruthenium complexes, standard glucose solutions for preparing calibration curves, and the necessary instructions for use for performing the assay.

10 The present invention also relates to glucose electrodes comprising a conductive material such as a conductive metal, especially platinum, copper, silver, aluminum, gold or carbon steel, such as vitreous carbon, carbon fibers, carbon nanotube fibers or diamond, etc., said conductive material is covered with a deposit comprising at least one GOx mutant according to the invention; said deposit also possibly comprising a redox polymer to improve the conductive properties of the conductive material.

15 The redox polymer is chosen from polymers based on ferrocene, osmium and ruthenium and conductive polymers such as polypyrrole and polyaniline.

20 The methods for immobilizing the GOx mutant on said conductive material may be chosen from the standard methods available to a person skilled in the art, which especially comprise inclusion of the GOx mutant in a polymer matrix, adsorption of the GOx mutant onto the surface of the polymer membrane, binding by covalent bonding or alternatively electrodeposition (Gao et al., Chem. Int. ED. 2002, 41, No. 5, 810-813).

25 Such electrodes are advantageously used in bioelectrical systems such as glucose biocells or glucose biosensors.

30 The present invention thus also relates to a glucose biosensor comprising an electrode according to the invention.

35 A glucose biosensor consists of an electrode on which is immobilized a bioreceptor that is capable of recognizing a biological target; the binding of the biological target to the bioreceptor leads to physicochemical modifications of the membrane and the production of an electrical signal via an electrochemical transducer (amperometric, potentiometric, 40 conductimetric, etc.) attached to the electrode; in the present case, the bioreceptor is a GOx mutant according to the invention and the biological target is its substrate: glucose.

45 According to an embodiment variant, the electrode on which is immobilized the GOx mutant is also covered with a membrane which prevents detachment of said mutant from the electrode. Said membrane may consist of nafion, cellulose or any biocompatible material, i.e. any material that is compatible with a physiological environment.

50 According to a variant of the invention, the glucose biosensor is implanted under the skin and allows the glucose concentration of the blood to be recorded.

55 The present invention also relates to biocells using glucose as a source of energy and comprising a first electrode according to the invention as anode and a second electrode as cathode. The cathode may be, for example, an enzymatic electrode for reducing oxygen, bearing an enzyme chosen from the class of copper-based enzymes (multicopper oxidases) and particularly bilirubine oxidase and laccase. It may also be a metal electrode, for example made of platinum, gold or a 60 platinum or gold alloy.

FIG. 1 more specifically illustrates an enzymatic glucose biocell; such an enzymatic biocell consists of two electrodes modified by immobilization of enzymes. A glucose oxidase (GOx) is attached to the anode (1) via a conductive polymer "I" and a bilirubine oxidase (BOD) is attached to the cathode (2) via a conductive polymer "II". When functioning, at the anode, the electrons are transferred from the glucose present

in the physiological fluid to the GOx, and then from the GOx to the conductive polymer "I" and from the conductive polymer "I" to the anode. At the cathode, the electrons are transferred from the cathode to the conductive polymer "II" and then to the BOD and finally from the BOD to the oxygen present in the physiological fluid.

It should be noted that a biocell may also optionally function by modifying the electrodes with their respective enzymes and by adding soluble mediators, such as ferrocenemethanol for the anode and potassium ferricyanide for the cathode, and by adding, where appropriate, a membrane separating the anode and the cathode.

The invention also relates to a process for assaying glucose in solution in a sample, comprising the following steps:

- a) introduction into said sample of a redox reagent whose reduction leads to a color change and of a GOx mutant according to the invention;
- b) measurement of the coloration intensity of the sample after enzymatic reaction;
- c) comparison of the coloration intensity measured in step b) with the intensity measured for standard solutions having a known glucose content;
- d) determination of the glucose concentration of said sample.

The redox reagent whose reduction leads to a color change is chosen from phenazinemethosulfate (PMS) in combination with 2,6-dichlorophenolindophenol (DCIP), potassium ferricyanide and ferrocene.

The invention also relates to a process for assaying the glucose of a sample, characterized in that it comprises the following steps:

- a) introduction into said sample of a glucose electrode according to the invention;
- b) measurement of the intensity of the current in the sample;
- c) comparison of the intensity of the current measured in step b) with the intensity measured for standard solutions having a known glucose content;
- d) determination of the glucose concentration of said sample.

Besides the preceding arrangements, the invention also comprises other arrangements that will emerge from the description that follows, which refer to examples of implementation of the present invention, and also to the attached figures, in which:

## FIGURES

FIG. 1 schematically represents a biocell.

FIG. 2 represents the plasmid map of the vector pET24a-GOx-penag-wt-His.

FIG. 3 is a graph illustrating the specific activity in U/mg of wild-type and mutant GOx from *Penicillium amagasakiense*.

FIG. 4 is a graph representing the ferrocenemethanol activity/oxygen activity ratio.

FIG. 5 shows a comparison of the specific activity of the wild-type and mutant GOx with glucose and xylose.

FIG. 6 is a graph representing the change in glucose oxidation current as a function of the glucose concentration.

FIG. 7 is a graph representing the change in the ratio of the glucose oxidation current under oxygen divided by the current under argon as a function of the glucose concentration.

## 1. MATERIALS

### 1.1 Bacterial Strains of *Escherichia coli*

DH<sub>5</sub>α:supE44, AlacU169, (Φ80 lacZDM15), hsdR17, recA1, endA1, gyrA96, thi-1, relA1 (Hanahan, 1983). This strain is used for the amplification of the plasmid during the steps of construction of the protein expression vectors. BL<sub>21</sub>:

F-ompT hsdSB(rB-, mB-) gal dcm (DE3) (Invitrogen). This strain is used for the production in conical flasks of GOx from *Penicillium amagasakiense* (penag). This strain is then transformed by the plasmid pET24a which contains the DNA sequence coding for the GOx of *Penicillium amagasakiense* under the dependence of the T7 promoter in the vector pET24a.

### 1.2 Vector

10 pET24a: plasmid pET24a containing the DNA sequence coding for the GOx of *Penicillium amagasakiense* cloned in phase with the C-terminal 6xHis label (the map of this plasmid is represented in FIG. 2).

### 1.3 Culture Medium

LB-rich medium:

Tryptone 10 g/1

Yeast extract 5 g/1

NaCl 5 g/1

Distilled H<sub>2</sub>O qsp 1 L

20 pH not adjusted, autoclaved for 50 minutes at 1 bar.

## 2. GENETIC ENGINEERING TECHNIQUES

### 2.1 Preparation of the Electrocompetent Bacteria

5 ml of DH5α cell preculture are inoculated in 1 L of LB and are cultured at 37° C. up to the exponential phase (OD between 0.6 and 0.8). The cells thus harvested by centrifugation at 4000 g are successively washed with cold milliQ® water until 2 ml of cells that have become electrocompetent are obtained.

### 2.2 Transformation of the Electrocompetent Bacteria

30 1 µl of plasmid DNA is incorporated into 40 µl of electrocompetent cells, placed in an electroporation tank and immediately transformed by the electroporator. 500 µl of SOC (culture medium containing 2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl<sub>2</sub>, 10 mM MgSO<sub>4</sub>) are added, incubated for 5 minutes in ice and cultured for 1 hour at 37° C. The 500 µl of cultures are then deposited in an LB agar dish and incubated overnight at 37° C.

### 2.3 Preparation of the DNA

40 This step is performed using the QIAprep® Miniprep kit (Qiagen) which makes it possible to extract and purify the plasmid DNA from 10 ml of culture of DH5α cells transformed with the desired plasmid. After collecting the cells by centrifugation, they undergo alkaline lysis, in the presence of

45 RNase, and also a precipitation of the genomic DNA with acetic acid. The DNA is then removed by centrifugation and the supernatant deposited on a column comprising a silica matrix, allowing selective adsorption of the plasmid DNA in the presence of a strong concentration of salt. After washing with ethanol to remove the salts, the RNA and the protein, the plasmid DNA is eluted with a buffer of weak ionic strength (water or buffer: Tris-HCl 10 mM pH 8.5). The DNA thus purified may be quantified by UV-visible spectrometry at 260 nm. An absorbance of 1 corresponds to a DNA concentration of 50 ng·µl<sup>-1</sup>. The purified plasmid DNA is stored at -20° C.

### 2.4 Digestion of the DNA

50 For total digestion, 200 to 500 ng of plasmid DNA are digested with 0.5 µl of XbaI restriction enzyme in the appropriate reaction buffer, in a final volume of 15 µl. The reaction takes place at 37° C. for 1 hour.

### 2.5 PCR-directed Mutagenesis

55 The GOx mutants V564I, V564S, V564T and V564S+K424E are obtained by directed mutagenesis. This method requires the use of a double-stranded plasmid (plasmid pET24a) bearing the gene of interest (GOx) and also 2 synthetic oligonucleotides whose sequence is complementary to the DNA strand to be modified, with the exception of the

desired mutation. These oligonucleotides contain between and 45 bases, with a melting point ( $T_m$ ) of greater than or equal to  $70^\circ\text{C}$ .

$$T_m = 81.5 + 0.41 (\% \text{ GC}) - 675/N - \% \text{ (not paired)}$$

With  $N$  the number of bases in the sequence, % GC the percentage of G and C bases in the sequence and % (not paired) the number of mutated bases (zero value in the case of deletion or insertion of a base). The chosen sequence must contain at least 40% of GC bases and must terminate with a C or a G.

The sequences of the oligonucleotides used are presented in table I above.

10 ng of parental plasmid, 12.5 ng of each of the primers, 1  $\mu\text{l}$  of a mixture of 10 mM concentrated dNTP, 5  $\mu\text{l}$  of reaction buffer, 1  $\mu\text{l}$  of Pfu Turbo DNA polymerase ( $2.5 \text{ U} \cdot \mu\text{l}^{-1}$ ) and 50  $\mu\text{l}$  qs of sterile water are mixed in a sterile Eppendorf flask.

The mutagenesis is performed via a sequence of temperature cycles performed automatically by a thermocycler. Each cycle comprises three steps. In a first stage, the 2 strands of the matrix DNA are separated by thermal denaturing, the oligonucleotides are then paired with their complementary sequence on the matrix DNA. They serve as primers for the elongation step, during which PfuTurbo polymerase (a heat-resistant DNA polymerase) synthesizes the DNA complementary to the parental strand.

Once this sequence of cycles is complete, the reaction product is treated with Dpn I, an endonuclease which specifically digests the methylated and hemimethylated DNA of the parental plasmid. The mutated DNA is finally introduced into competent cells which link the ends of the plasmid that are still free after the DNA synthesis.

#### 2.6 Sequencing of the Double-stranded DNA

The double-stranded DNA is sequenced with the genomics platform of the université Victor Segalen. The sequence reactions are performed with the BigDye Terminator v.1.1 or v3.1 sequencing kit. The reagent contains the four ddNTPs with different fluorescent markers (BigDye Terminators), AmpliTaq DNA polymerase, and all the other components necessary for the reaction. The extension products must be purified before passage on the ABI 3130x1 sequencer, to remove the markers not incorporated, salts and other contaminants.

#### 3. Production And Purification Of The Glucose Oxidase Enzyme Of *Penicillium Amagasakiense*

##### 3.1 Production of the Wild-type and Mutated GOx Enzymes

The GOx enzyme is produced in the strain *E. coli* BL21 via the recombinant plasmid pET24a bearing the sequence coding for the wild-type or mutated GOx. A preculture of 2 ml of LB medium supplemented with kanamycin (1 $\times$ ) is seeded with an isolated clone on an LB agar dish supplemented with kanamycin (1 $\times$ ) and left stirring at 220 rpm overnight at  $37^\circ\text{C}$ . A 50 ml culture is then seeded at 1/25 in LB medium supplemented with kanamycin (1 $\times$ ) in a 250 ml conical flask. This flask is incubated at  $37^\circ\text{C}$ . with stirring (220 rpm) to an  $\text{OD}_{600 \text{ nm}}$  of between 0.8 and 1  $\text{OD}_{600 \text{ nm}}/\text{ml}$ . The culture is then induced with 500  $\mu\text{M}$  of IPTG and then left stirring (220 rpm) at  $37^\circ\text{C}$ . for 2 hours.

##### 3.2 Preparation of the Soluble Extracts

The cells harvested by centrifugation (4500 g,  $4^\circ\text{C}$ ) are first washed in 5 ml of Tris/HCl 20 mM buffer; NaCl 100 mM; EDTA 1 mM. The cells harvested by centrifugation (4500 g,  $4^\circ\text{C}$ ) are then washed in 5 ml of Tris/HCl 20 mM buffer; NaCl 100 mM; EDTA 1 mM containing 3 M urea in order to embrittle the cell membrane. The harvested cells (4500 g,  $4^\circ\text{C}$ ) are incubated for 1 hour on ice in the presence of 5 ml of Tris/HCl 20 mM buffer; NaCl 100 mM; EDTA 1 mM con-

taining 8M urea allowing complete lysis of the cells. The supernatant is harvested after centrifugation at 4500 g at  $4^\circ\text{C}$ . and then stored at  $-20^\circ\text{C}$ .

##### 3.3 Reconstitution of the GOx

In the bacterium *E. coli* BL21, glucose oxidase from *Penicillium amagasakiense* is overexpressed in its Apo form, i.e. in the absence of its cofactor flavine adenine dinucleotide (FAD). It is thus necessary to reconstitute it chemically. To do this, the 5 ml of soluble extract obtained are added dropwise with vigorous stirring, so as to avoid the precipitation of the protein, to 500 ml of a reconstitution solution containing 10% glycerol, 1 mM of reduced glutathione, 1 mM of oxidized glutathione, 100  $\mu\text{M}$  of FAD in Tris/HCl 20 mM pH 8 buffer. This solution is stored for 5 days at  $4^\circ\text{C}$ . protected from light.

##### 3.4 Purification of the GOx

###### Anion-exchange Chromatography

The reconstitution solution, dialyzed in 20 mM pH 6 acetate buffer to allow precipitation of the remaining Apo form, is concentrated to 5 ml on an Amicon YM10 membrane 20 and then filtered through a 0.22  $\mu\text{m}$  filter. This solution is injected onto a QFF anion-exchange column (GE Healthcare®), coupled to the AKTA purifier system (GE Healthcare®) equilibrated in a 20 mM pH 6 sodium acetate buffer. The elution is performed with a gradient of from 0% to 30% 25 of a 50 mM sodium acetate, 250 mM NaCl, pH 3 buffer at a flow rate of 1 ml/min. The fractions containing the GOx protein are identified by an ABTS activity test and are combined, concentrated and desalinated with a 100 mM pH 5 phosphate buffer by centrifugation on an Amicon YM10 30 membrane. At this stage, the GOx protein is pure and can be stored at  $4^\circ\text{C}$ . in soluble form.

#### 4. Characterization of the wild-type and mutated gox Enzymes

##### 4.1 Measurement of the Concentration

The protein concentration determination is performed by UV-visible spectroscopy using a Varian spectrophotometer in a 100 mM pH 5 phosphate buffer at  $25^\circ\text{C}$ . The purified GOx proteins have a characteristic spectrum between 200 and 800 nm. The first band at 280 nm is characteristic of the absorption 40 of the aromatic amino acids and makes it possible to obtain the enzyme concentration ( $\epsilon=263 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ ). The other two bands at 380 and 461 nm are characteristic of the FAD integrated into the protein.

The absorbance value at 461 nm makes it possible to obtain 45 the concentration of cofactor present in the protein ( $\epsilon=12.83 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ ). The ratio between the GOx concentration and the FAD concentration is equal to 2 when the protein is completely reconstituted.

##### 4.2 Enzymatic Test

The enzymatic tests are performed by UV-visible spectroscopy using a Varian spectrophotometer in air.

The enzymatic tests are performed in the presence of a strong excess of glucose (150 mM) so as to be able to observe only the reoxidation of GOx by the mediators.

55 Two enzymatic tests are performed. The first with an ABTS-HRP mixture (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)-horseradish peroxidase) to be able to observe the reoxidation with oxygen. The ABTS is oxidized in the presence of HRP and  $\text{H}_2\text{O}_2$ . And the second with oxidized ferrocenemethanol, so as to be able to observe the reoxidation of the GOx by a redox mediator having a redox potential close to that used in electrochemistry.

##### 4.2.1 ABTS-HRP Enzymatic Test

The tests are performed in a 100 mM pH 5 phosphate buffer 60 at  $37^\circ\text{C}$ . in a volume of 3 ml containing 100  $\mu\text{l}$  of HRP (60 U/ml), 24  $\mu\text{l}$  of ABTS (11.5 mg/ml) and 150 mM of glucose. The ABTS oxidation is monitored at 405 nm as a function of

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time ( $\epsilon=36.8 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ ). The specific activity of the enzyme is expressed as micromoles of product appeared per mg of enzyme (U/mg). The enzyme is diluted to 50 nM so as to measure a slope of between 0.05 and 0.3 OD<sub>405 nm</sub>/min.

**4.2.2 Ferrocenemethanol Enzymatic Test (FMox)**  
**4.2.2.1 Preparation of the Ferrocenemethanol**

Commercial ferrocenemethanol is in reduced form, and it is thus necessary to oxidize it in order to be able to use it in the enzymatic tests. To do this, 100 mg of reduced ferrocenemethanol are dissolved in 50 ml of 50 mM pH 7.5 phosphate buffer and placed in an electrolysis cell comprising a working electrode (carbon electrode), an Ag/AgCl reference electrode, a platinum counterelectrode placed in a sinter containing 50 mM pH 7.5 phosphate buffer. The system is under argon. The electrolysis is performed at 0.5 V for 4 hours.

**4.2.2.2 Enzymatic Test**

The tests are performed in a 50 mM pH 7.5 phosphate buffer at 37°C. in a volume of 3 ml containing 1 mM of FMox and 150 mM glucose. The ferrocenemethanol reduction is monitored at 625 nm as a function of time ( $\epsilon=0.413 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ ) The specific activity of the enzyme is expressed in micromoles of product appeared per mg of enzyme (U/mg) (FIG. 3). The enzyme is diluted to 50 nM so as to be able to measure a slope of between 0.001 and 0.1 OD<sub>625 nm</sub>/min.

It is noted that the four mutants show a marked decrease in activity toward oxygen relative to the wild-type GOx.

The activity toward ferrocenemethanol is different depending on the mutant. Thus, the V564S mutant has a lower

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activity, the V564I mutant has a similar activity, whereas the V564T mutant shows a strong increase in activity relative to the wild-type GOx.

The FMox activity/ABTS activity ratio makes it possible to obtain the specificity of the enzyme toward the substrate (ABTS or FMox). The greater the ratio, the less sensitive the enzyme is to oxygen (FIG. 4).

It is noted that the mutants V564S and V564T are very insensitive to oxygen, which makes them good candidates for use in electrochemical systems.

The same enzymatic tests were performed in the presence of xylose (FIG. 5): the ABTS and FMox activities for xylose are very low compared with the activities for glucose. Xylose does not come into competition with glucose as a substrate for the wild-type and mutant glucose oxidases of *Penicillium amagasakiense*.

**5. Electrochemical Tests**

By way of example, FIG. 6 represents the change in the glucose oxidation current as a function of the glucose concentration in a PBS buffer under argon and at 37°C. Each electrode is composed of 5% by mass of glucose oxidase, 10% by mass of crosslinking agent and 75% by mass of redox polymer. FIG. 7 represents the change in the ratio of the glucose oxidation current under oxygen divided by the current under argon as a function of the glucose concentration. This figure clearly shows a decrease in the effect of oxygen on the glucose oxidation when the mutated enzymes are used.

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ccagaatgct	actctttccc	agtggtcgga	ttatgtctta	cagaacttcc	gtcccaactg	1560
geatgctgt	agcagctgt	ctatgatgtc	tagagagott	ggtgggtgtc	ttgatgtac	1620
tgccaaagg	tacggtaccc	aaggcctacg	tgtcattgac	gggtctatcc	ctccgactca	1680
ggtgtcttcc	cattccatga	ccatTTcta	cggaatggct	ttgaagggtg	ctgatgccc	1740
tttggatgac	tatgcca	aaa	gtgcctcgct			1770

<210> SEQ ID NO 4  
<211> LENGTH: 587  
<212> TYPE: PRT  
<213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 4

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Tyr Leu Pro Ala Gln Gln Ile Asp Val Gln Ser Ser Leu Leu Ser Asp  
 1 5 10 15

Pro Ser Lys Val Ala Gly Lys Thr Tyr Asp Tyr Ile Ile Ala Gly Gly  
 20 25 30

Gly Leu Thr Gly Leu Thr Val Ala Ala Lys Leu Thr Glu Asn Pro Lys  
 35 40 45

Ile Lys Val Leu Val Ile Glu Lys Gly Phe Tyr Glu Ser Asn Asp Gly  
 50 55 60

Ala Ile Ile Glu Asp Pro Asn Ala Tyr Gly Gln Ile Phe Gly Thr Thr  
 65 70 75 80

Val Asp Gln Asn Tyr Leu Thr Val Pro Leu Ile Asn Asn Pro Thr Asn  
 85 90 95

Asn Ile Lys Ala Gly Lys Gly Leu Gly Ser Thr Leu Ile Asn Gly  
 100 105 110

Asp Ser Trp Thr Arg Pro Asp Lys Val Gln Ile Asp Ser Trp Glu Lys  
 115 120 125

Val Phe Gly Met Glu Gly Trp Asn Trp Asp Asn Met Phe Glu Tyr Met  
 130 135 140

Lys Lys Ala Glu Ala Ala Arg Thr Pro Thr Ala Ala Gln Leu Ala Ala  
 145 150 155 160

Gly His Ser Phe Asn Pro Thr Cys His Gly Thr Asn Pro Thr Val Gln  
 165 170 175

Ser Gly Ala Arg Asp Asn Gly Gln Pro Trp Ser Pro Ile Met Lys Ala  
 180 185 190

Leu Met Asn Thr Val Ser Ala Leu Gly Val Pro Val Gln Gln Asp Phe  
 195 200 205

Leu Cys Gly His Pro Arg Gly Val Ser Met Ile Met Asn Asn Leu Asp  
 210 215 220

Glu Asn Gln Val Arg Val Asp Ala Ala Arg Ala Trp Leu Leu Pro Asn  
 225 230 235 240

Tyr Gln Arg Ser Asn Leu Glu Ile Leu Thr Gly Gln Met Val Gly Lys  
 245 250 255

Val Leu Phe Lys Gln Thr Ala Ser Gly Pro Gln Ala Val Gly Val Asn  
 260 265 270

Phe Gly Thr Asn Lys Ala Val Asn Phe Asp Val Phe Ala Lys His Glu  
 275 280 285

Val Leu Leu Ala Ala Gly Ser Ala Ile Ser Pro Leu Ile Leu Glu Tyr  
 290 295 300

Ser Gly Ile Gly Leu Lys Ser Val Leu Asp Gln Ala Asn Pro Thr Gln  
 305 310 315 320

Leu Leu Asp Leu Pro Val Gly Ile Asn Met Gln Asp Gln Thr Thr Thr  
 325 330 335

Thr Val Ser Ser Arg Ala Ser Ser Ala Gly Ala Gly Gln Gly Gln Ala  
 340 345 350

Val Phe Phe Ala Asn Pro Thr Glu Thr Phe Gly Asp Tyr Ala Pro Gln  
 355 360 365

Ala Arg Asp Leu Leu Asn Thr Lys Leu Asp Gln Trp Ala Glu Glu Thr  
 370 375 380

Val Ala Arg Gly Gly Phe His Asn Pro Thr Ala Leu Lys Val Gln Tyr  
 385 390 395 400

Glu Asn Tyr Arg Asn Trp Leu Leu Asp Glu Asp Val Ala Phe Ala Glu  
 405 410 415

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Leu Phe Met Asp Thr Glu Gly Lys Ile Asn Phe Asp Leu Trp Asp Leu  
 420 425 430  
 Ile Pro Phe Thr Arg Gly Ser Val His Ile Leu Ser Ser Asp Pro Tyr  
 435 440 445  
 Leu Trp Gln Phe Ala Asn Asp Pro Lys Phe Phe Leu Asn Glu Phe Asp  
 450 455 460  
 Leu Leu Gly Gln Ala Ala Ala Ser Lys Leu Ala Arg Asp Leu Thr Ser  
 465 470 475 480  
 Gln Gly Ala Met Lys Glu Tyr Phe Ala Gly Glu Thr Leu Pro Gly Tyr  
 485 490 495  
 Asn Leu Val Gln Asn Pro Thr Leu Ser Gln Trp Ser Asp Tyr Val Leu  
 500 505 510  
 Gln Asn Phe Arg Pro Asn Trp His Ala Val Ser Ser Cys Ser Met Met  
 515 520 525  
 Ser Arg Glu Leu Gly Gly Val Val Asp Ala Thr Ala Lys Val Tyr Gly  
 530 535 540  
 Thr Gln Gly Leu Arg Val Ile Asp Gly Ser Ile Pro Pro Thr Gln Val  
 545 550 555 560  
 Ser Ser His Ser Met Thr Ile Phe Tyr Gly Met Ala Leu Lys Val Ala  
 565 570 575  
 Asp Ala Ile Leu Asp Asp Tyr Ala Lys Ser Ala  
 580 585

<210> SEQ ID NO 5  
 <211> LENGTH: 1768  
 <212> TYPE: DNA  
 <213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 5

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tgctgc当地 ttgacagaaa accccaagat caaaagtccgt gtcatggaaa agggcttcta  180
tgatgtccac gatggggcca tcatcgatgg tccaaatgtc tatggacaaa tctttggcac  240
cactgttgc acagaactacc tcaccgttcc cctgatcaac aaccgcacga acaatataa  300
ggccggtaaa ggtcttggag atcaaccttg ataaacgggtg actcctggac tcgcccagac  360
aaagtccaga ttgattctt ggagaaggtc tttggcatga aggttggaaat tgggacaaca  420
tgttcgatg catgaaagaag gccggggctg cacgtacccc tactgtgtc cagttgtc  480
ctggccactc cttcaatgtc acctgcccattt gaaccaacgg tactgttcaa tccggagccc 540
gtgacaacgg ccagccttgg tctccttata tgaaggccct tatgaacacc gtctcgcccc 600
ttgggttccc cgtacagcaa gactttctt gtggatcc acgggggttc tctatgtca  660
tgaacaatct cgacgaaaac caagttcgatgg ttgatgtc ccgtgcattt ctgttccca 720
actaccagcg ctcgaattttt gagatccttca ctggatcgat ggttggaaag gttctgttta 780
aacagaccgc atccggtccc caggctgtt ggttgcactt cgggtactaat aaggccgtca 840
actttgacgt ctttgcataag catggggcc ttttgggtgc tgggtcactt atctctccgc 900
tgatcttggatattctggc ataggcttga agtctgttct tgatcaagcc aatgtcactc 960
agcttcttgc tcttcttgc ggttatcaata tgcaagatca gaccacaacc actgtcagg 1020
cccggtcttagt ttccgggtt gctgggtcagg gtcaggccgt cttcttcgaa aatttcactg 1080
agaccccttcgg tgactacgccc ccccaaggcca gggacttactt caacaccaag ctcgaccaat 1140
  
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ggcccgagga gaccgttgcg cgccgtgggtt tccataatgt aactgctc aaagtacaat 1200
acaaaaacta tcgttaactgg ctccttgacg aagacgtcgc ctccggag ctttcatgg 1260
acaccgaggg caagatcaac ttgcatttat gggatctcat cccttcaact cgtggttccg 1320
tccatatacct cagtagcgat ctttacctat ggcaattcgc caacgacccc aaattctcc 1380
tgaacgagtt tgacccctt ggtcaagctg ccgcattccaa gcttgcgt gatctacta 1440
gccaaggcgc tatgaaggag tacttcgcgg gggagactct tccaggatac aacttggtcc 1500
agaatgtac tcttcccaag tggtcggatt atgtcttaca gaacttccgt cccaaactggc 1560
atgcgtgtgag cagctgctct atgatgtcta gagagcttgg tgggtgcgtt gatgtactg 1620
ccaagggtgta cggtaaccaa ggcctacgtg tcattgacgg gtctattcct ccgactcagg 1680
tgtcttccca taccatgacc atttctacg gaatggctt gaagggtgct gatgccattt 1740
tggatgacta tgccaaaagt gcctcgct 1768

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&lt;210&gt; SEQ\_ID NO 6

&lt;211&gt; LENGTH: 587

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 6

Tyr	Leu	Pro	Ala	Gln	Gln	Ile	Asp	Val	Gln	Ser	Ser	Leu	Leu	Ser	Asp
1				5				10				15			

Pro	Ser	Lys	Val	Ala	Gly	Lys	Thr	Tyr	Asp	Tyr	Ile	Ile	Ala	Gly	Gly
		20				25					30				

Gly	Leu	Thr	Gly	Leu	Thr	Val	Ala	Ala	Lys	Leu	Thr	Glu	Asn	Pro	Lys
	35			40					45						

Ile	Lys	Val	Leu	Val	Ile	Glu	Lys	Gly	Phe	Tyr	Glu	Ser	Asn	Asp	Gly
	50				55				60						

Ala	Ile	Ile	Glu	Asp	Pro	Asn	Ala	Tyr	Gly	Gln	Ile	Phe	Gly	Thr	Thr
65				70				75			80				

Val	Asp	Gln	Asn	Tyr	Leu	Thr	Val	Pro	Leu	Ile	Asn	Asn	Pro	Thr	Asn
	85				90				95						

Asn	Ile	Lys	Ala	Gly	Lys	Gly	Leu	Gly	Gly	Ser	Thr	Leu	Ile	Asn	Gly
	100				105				110						

Asp	Ser	Trp	Thr	Arg	Pro	Asp	Lys	Val	Gln	Ile	Asp	Ser	Trp	Glu	Lys
	115				120				125						

Val	Phe	Gly	Met	Glu	Gly	Trp	Asn	Trp	Asp	Asn	Met	Phe	Glu	Tyr	Met
	130			135				140							

Lys	Lys	Ala	Glu	Ala	Ala	Arg	Thr	Pro	Thr	Ala	Ala	Gln	Leu	Ala	Ala
145					150				155			160			

Gly	His	Ser	Phe	Asn	Pro	Thr	Cys	His	Gly	Thr	Asn	Pro	Thr	Val	Gln
	165				170				175						

Ser	Gly	Ala	Arg	Asp	Asn	Gly	Gln	Pro	Trp	Ser	Pro	Ile	Met	Lys	Ala
	180				185				190						

Leu	Met	Asn	Thr	Val	Ser	Ala	Leu	Gly	Val	Pro	Val	Gln	Gln	Asp	Phe
	195				200				205						

Leu	Cys	Gly	His	Pro	Arg	Gly	Val	Ser	Met	Ile	Met	Asn	Asn	Leu	Asp
	210				215				220						

Glu	Asn	Gln	Val	Arg	Val	Asp	Ala	Ala	Arg	Ala	Trp	Leu	Leu	Pro	Asn
225			230					235			240				

Tyr	Gln	Arg	Ser	Asn	Leu	Glu	Ile	Leu	Thr	Gly	Gln	Met	Val	Gly	Lys
	245				250				255						

Val	Leu	Phe	Lys	Gln	Thr	Ala	Ser	Gly	Pro	Gln	Ala	Val	Gly	Val	Asn
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260	265	270
Phe Gly Thr Asn Lys Ala Val Asn Phe Asp Val Phe Ala Lys His Glu		
275	280	285
Val Leu Leu Ala Ala Gly Ser Ala Ile Ser Pro Leu Ile Leu Glu Tyr		
290	295	300
Ser Gly Ile Gly Leu Lys Ser Val Leu Asp Gln Ala Asn Pro Thr Gln		
305	310	315
Leu Leu Asp Leu Pro Val Gly Ile Asn Met Gln Asp Gln Thr Thr Thr		
325	330	335
Thr Val Ser Ser Arg Ala Ser Ser Ala Gly Ala Gly Gln Gly Gln Ala		
340	345	350
Val Phe Phe Ala Asn Pro Thr Glu Thr Phe Gly Asp Tyr Ala Pro Gln		
355	360	365
Ala Arg Asp Leu Leu Asn Thr Lys Leu Asp Gln Trp Ala Glu Glu Thr		
370	375	380
Val Ala Arg Gly Phe His Asn Pro Thr Ala Leu Lys Val Gln Tyr		
385	390	395
Glu Asn Tyr Arg Asn Trp Leu Leu Asp Glu Asp Val Ala Phe Ala Glu		
405	410	415
Leu Phe Met Asp Thr Glu Gly Lys Ile Asn Phe Asp Leu Trp Asp Leu		
420	425	430
Ile Pro Phe Thr Arg Gly Ser Val His Ile Leu Ser Ser Asp Pro Tyr		
435	440	445
Leu Trp Gln Phe Ala Asn Asp Pro Lys Phe Phe Leu Asn Glu Phe Asp		
450	455	460
Leu Leu Gly Gln Ala Ala Ala Ser Lys Leu Ala Arg Asp Leu Thr Ser		
465	470	475
Gln Gly Ala Met Lys Glu Tyr Phe Ala Gly Glu Thr Leu Pro Gly Tyr		
485	490	495
Asn Leu Val Gln Asn Pro Thr Leu Ser Gln Trp Ser Asp Tyr Val Leu		
500	505	510
Gln Asn Phe Arg Pro Asn Trp His Ala Val Ser Ser Cys Ser Met Met		
515	520	525
Ser Arg Glu Leu Gly Gly Val Val Asp Ala Thr Ala Lys Val Tyr Gly		
530	535	540
Thr Gln Gly Leu Arg Val Ile Asp Gly Ser Ile Pro Pro Thr Gln Val		
545	550	555
Ser Ser His Thr Met Thr Ile Phe Tyr Gly Met Ala Leu Lys Val Ala		
565	570	575
Asp Ala Ile Leu Asp Asp Tyr Ala Lys Ser Ala		
580	585	

&lt;210&gt; SEQ ID NO 7

&lt;211&gt; LENGTH: 1770

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 7

tatgtacctg cctgccccaaac agattgtatgt ccagtctagt cttctcagtg acccttagcaa	60
ggttgcagga aagacctatg attacatcat tgctggtggt ggtttgactg gccttactgt	120
tgctgccaaa ttgacagaaaa accccaagat caaagtctgt gtcattgaaa agggcttcta	180
tgagtccaaac gatggagcca tcatcgagga tccaaatgct tatggacaaa tctttggcac	240
cactgttgac cagaactacc tcaccgttcc cctgatcaac aaccgcacga acaatatcaa	300

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ggccggtaaa ggtcttggag gatcaacctt gataaacggg gactcctgga ctcgcccaga 360  
 caaaggccag attgattctt gggagaaggt ctttggcatg gaagggtgga attgggacaa 420  
 catgttcgag tacatgaaga aggccgaggc tgcacgtacc cctactgctg ctcagcttc 480  
 tgctggcac tccttcaatg ctacctgcca tggAACCAAC ggtactgttc aatccggagc 540  
 ccgtgacaac ggccagcctt ggtctcctat tatgaaggcc cttatgaaca ccgtctcggc 600  
 ccttgggtc cccgtacagc aagactttct ctgtggatcat ccacgagggtg tctctatgtat 660  
 catgaacaat ctgcacgaaa accaagtccg tggatgtcat gcccgtgcat ggctgcttc 720  
 caactaccag cgctcgaatt tggagatct tactggtcag atgggtggaa aggttctgtt 780  
 taaacagacc gcacccggc cccaggctgt tggatgtgaac ttccgtacta ataaggccgt 840  
 caactttgac gtctttgcta agcatgaggt cctttggct gctggctcaag ctatctcc 900  
 gctgatcttgaatattctg gcataggctt gaagtctgtt cttgatcaag ccaatgtcac 960  
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 tgagacccctc ggtgactacg ccccccaggc cagggactta ctcaacacca agctcgacca 1140  
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 atacgaaaac tatacgtaact ggctcccttga cgaagacgtc gcttgcggc agctttcat 1260  
 ggacaccggag ggcaagatca acttcgattt atggatctc atcccttca ctccgtggtc 1320  
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 cctgaacccggat tttggatcttcc ttggatgttcaagc tgccgttcc aagcttgcgtc gtatctcac 1440  
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 ccagaatgtc actctttccc agtggatgttcaat cagaacttcc gtcggacttgc 1560  
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 tgccaaagggtg tacggatccc aaggccatcg tgcatttgc gggatgttcc ctccgactca 1680  
 ggtgttccatattatga ccattttcttca cggaaatggct ttggatgttgc ctgtatccat 1740  
 ttggatgttcaat cggccaaaaa gtgcctcgct 1770

&lt;210&gt; SEQ ID NO 8

&lt;211&gt; LENGTH: 587

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 8

Tyr	Leu	Pro	Ala	Gln	Gln	Ile	Asp	Val	Gln	Ser	Ser	Leu	Leu	Ser	Asp
1				5				10				15			

Pro	Ser	Lys	Val	Ala	Gly	Lys	Thr	Tyr	Asp	Tyr	Ile	Ile	Ala	Gly	Gly
						20		25				30			

Gly	Leu	Thr	Gly	Leu	Thr	Val	Ala	Ala	Lys	Leu	Thr	Glu	Asn	Pro	Lys
						35		40			45				

Ile	Lys	Val	Leu	Val	Ile	Glu	Lys	Gly	Phe	Tyr	Glu	Ser	Asn	Asp	Gly
						50		55			60				

Ala	Ile	Ile	Glu	Asp	Pro	Asn	Ala	Tyr	Gly	Gln	Ile	Phe	Gly	Thr	Thr
						65		70		75		80			

Val	Asp	Gln	Asn	Tyr	Leu	Thr	Val	Pro	Leu	Ile	Asn	Asn	Pro	Thr	Asn
							85		90		95				

Asn	Ile	Lys	Ala	Gly	Lys	Gly	Leu	Gly	Ser	Thr	Leu	Ile	Asn	Gly
							100		105		110			

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Asp Ser Trp Thr Arg Pro Asp Lys Val Gln Ile Asp Ser Trp Glu Lys  
 115 120 125  
 Val Phe Gly Met Glu Gly Trp Asn Trp Asp Asn Met Phe Glu Tyr Met  
 130 135 140  
 Lys Lys Ala Glu Ala Ala Arg Thr Pro Thr Ala Ala Gln Leu Ala Ala  
 145 150 155 160  
 Gly His Ser Phe Asn Pro Thr Cys His Gly Thr Asn Pro Thr Val Gln  
 165 170 175  
 Ser Gly Ala Arg Asp Asn Gly Gln Pro Trp Ser Pro Ile Met Lys Ala  
 180 185 190  
 Leu Met Asn Thr Val Ser Ala Leu Gly Val Pro Val Gln Gln Asp Phe  
 195 200 205  
 Leu Cys Gly His Pro Arg Gly Val Ser Met Ile Met Asn Asn Leu Asp  
 210 215 220  
 Glu Asn Gln Val Arg Val Asp Ala Ala Arg Ala Trp Leu Leu Pro Asn  
 225 230 235 240  
 Tyr Gln Arg Ser Asn Leu Glu Ile Leu Thr Gly Gln Met Val Gly Lys  
 245 250 255  
 Val Leu Phe Lys Gln Thr Ala Ser Gly Pro Gln Ala Val Gly Val Asn  
 260 265 270  
 Phe Gly Thr Asn Lys Ala Val Asn Phe Asp Val Phe Ala Lys His Glu  
 275 280 285  
 Val Leu Leu Ala Ala Gly Ser Ala Ile Ser Pro Leu Ile Leu Glu Tyr  
 290 295 300  
 Ser Gly Ile Gly Leu Lys Ser Val Leu Asp Gln Ala Asn Pro Thr Gln  
 305 310 315 320  
 Leu Leu Asp Leu Pro Val Gly Ile Asn Met Gln Asp Gln Thr Thr Thr  
 325 330 335  
 Thr Val Ser Ser Arg Ala Ser Ser Ala Gly Ala Gly Gln Gly Gln Ala  
 340 345 350  
 Val Phe Phe Ala Asn Pro Thr Glu Thr Phe Gly Asp Tyr Ala Pro Gln  
 355 360 365  
 Ala Arg Asp Leu Leu Asn Thr Lys Leu Asp Gln Trp Ala Glu Glu Thr  
 370 375 380  
 Val Ala Arg Gly Phe His Asn Pro Thr Ala Leu Lys Val Gln Tyr  
 385 390 395 400  
 Glu Asn Tyr Arg Asn Trp Leu Leu Asp Glu Asp Val Ala Phe Ala Glu  
 405 410 415  
 Leu Phe Met Asp Thr Glu Gly Lys Ile Asn Phe Asp Leu Trp Asp Leu  
 420 425 430  
 Ile Pro Phe Thr Arg Gly Ser Val His Ile Leu Ser Ser Asp Pro Tyr  
 435 440 445  
 Leu Trp Gln Phe Ala Asn Asp Pro Lys Phe Phe Leu Asn Glu Phe Asp  
 450 455 460  
 Leu Leu Gly Gln Ala Ala Ala Ser Lys Leu Ala Arg Asp Leu Thr Ser  
 465 470 475 480  
 Gln Gly Ala Met Lys Glu Tyr Phe Ala Gly Glu Thr Leu Pro Gly Tyr  
 485 490 495  
 Asn Leu Val Gln Asn Pro Thr Leu Ser Gln Trp Ser Asp Tyr Val Leu  
 500 505 510  
 Gln Asn Phe Arg Pro Asn Trp His Ala Val Ser Ser Cys Ser Met Met  
 515 520 525

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Ser Arg Glu Leu Gly Gly Val Val Asp Ala Thr Ala Lys Val Tyr Gly  
530 535 540

Thr Gln Gly Leu Arg Val Ile Asp Gly Ser Ile Pro Pro Thr Gln Val  
545 550 555 560

Ser Ser His Ile Met Thr Ile Phe Tyr Gly Met Ala Leu Lys Val Ala  
565 570 575

Asp Ala Ile Leu Asp Asp Tyr Ala Lys Ser Ala  
580 585

<210> SEQ ID NO 9

<211> LENGTH: 1770

<212> TYPE: DNA

<213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 9

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tgcgtccaaa	ttgacagaaaa	accccaagat	caaagtctgt	gtcattgaaa	agggtttctta	180
tgagtcacac	gatggggcca	tcatcgagga	tccaaatgt	tatggacaaa	tctttggcac	240
cactgttgcac	cagaactacc	tcaccgttcc	cctgatcaac	aaccgcacga	acaatatcaa	300
ggcccgtaaa	ggtcttggag	gatcaacctt	gataaacgggt	gactcctgga	ctcgcccaga	360
caaagtccag	attgattctt	gggagaaggt	cttggcatg	gaagggttgg	attgggacaa	420
catgttcgag	tatcatgaaga	aggccgaggc	tgcacgtacc	cctactgtgt	ctcagctgc	480
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ccttgggttc	cccgatcagc	aagactttct	ctgtggtcat	ccacgagggt	tctctatgat	660
catgaacaat	ctcgacgaaa	accaagttcg	tgttgatgt	gcccgtgtc	ggctgcttcc	720
caactaccag	cgctcgaatt	tggagatct	tactggtag	atgggttggaa	aggttctgtt	780
taaacagacc	gcatccggtc	cccaggctgt	tggtgtgaac	ttcggacta	ataaggccgt	840
caactttgac	gtctttgcta	agcatgaggt	ccttttggct	gtgggttcag	ctatctctcc	900
gtgtatcttgc	aatatttctg	gcataaggctt	gaagtctgtt	cttgatcaag	ccaatgtcac	960
tcagcttcttgc	gatcttccttgc	ttggtatcaa	tatgcaagat	cagaccacaa	ccactgtcac	1020
ttcccgtgtc	agttccgcttgc	gtgtggtca	gggtcaggcc	gtcttcttgc	ccaatttcac	1080
tgagacccatc	ggtgactacg	ccccccagggc	cagggactta	ctcaacacca	agctcgacca	1140
atggggccgag	gagaccgttg	cgcgcgggtgg	tttccataat	gtaaactgtc	tcaaagtaca	1200
atacgaaaac	tatcgtaact	ggctcccttga	cgaagacgtc	gccttcgccc	agctttcat	1260
ggacacccgag	ggcgagatca	acttcgattt	atgggatctc	atccctttca	ctcggttgc	1320
cgtccatatac	tcagtagcg	atccttacct	atggcaattc	gcacaacgacc	ccaaatttctt	1380
cctgaacccgag	tttgcaccc	ttggtcaagc	tgccgcttcc	aagcttgc	gtgatctcac	1440
tagccaaaggc	gctatgaagg	agtacttcgc	cggggagact	cttccaggat	acaacttggt	1500
ccagaatgtc	actctttccc	agtggtcgga	ttatgttta	cagaacttcc	gtcccaactg	1560
gcgtgtgtgc	agcagactgt	ctatgtatgc	tagagagctt	ggtgggtgtc	ttgatgtac	1620
tgccaaagggt	tacggtaccc	aaggcctacg	tgtcattgac	gggtcttattc	ctccgactca	1680
ggtgtcttcc	cattccatga	ccatccatga	cggaatggct	ttgaagggtg	ctgatgcac	1740
tttggatgac	tatgccaaa	gtgcctcgct				1770

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<210> SEQ\_ID NO 10  
<211> LENGTH: 587  
<212> TYPE: PRT  
<213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 10

Tyr	Leu	Pro	Ala	Gln	Gln	Ile	Asp	Val	Gln	Ser	Ser	Leu	Leu	Ser	Asp
1				5				10				15			
Pro	Ser	Lys	Val	Ala	Gly	Lys	Thr	Tyr	Asp	Tyr	Ile	Ile	Ala	Gly	Gly
	20					25					30				
Gly	Leu	Thr	Gly	Leu	Thr	Val	Ala	Ala	Lys	Leu	Thr	Glu	Asn	Pro	Lys
	35					40				45					
Ile	Lys	Val	Leu	Val	Ile	Glu	Lys	Gly	Phe	Tyr	Glu	Ser	Asn	Asp	Gly
	50				55				60						
Ala	Ile	Ile	Glu	Asp	Pro	Asn	Ala	Tyr	Gly	Gln	Ile	Phe	Gly	Thr	Thr
	65				70				75		80				
Val	Asp	Gln	Asn	Tyr	Leu	Thr	Val	Pro	Leu	Ile	Asn	Asn	Pro	Thr	Asn
	85					90				95					
Asn	Ile	Lys	Ala	Gly	Lys	Gly	Leu	Gly	Gly	Ser	Thr	Leu	Ile	Asn	Gly
	100					105				110					
Asp	Ser	Trp	Thr	Arg	Pro	Asp	Lys	Val	Gln	Ile	Asp	Ser	Trp	Glu	Lys
	115					120				125					
Val	Phe	Gly	Met	Glu	Gly	Trp	Asn	Trp	Asp	Asn	Met	Phe	Glu	Tyr	Met
	130					135				140					
Lys	Lys	Ala	Glu	Ala	Ala	Arg	Thr	Pro	Thr	Ala	Ala	Gln	Leu	Ala	Ala
	145				150				155		160				
Gly	His	Ser	Phe	Asn	Pro	Thr	Cys	His	Gly	Thr	Asn	Pro	Thr	Val	Gln
	165					170				175					
Ser	Gly	Ala	Arg	Asp	Asn	Gly	Gln	Pro	Trp	Ser	Pro	Ile	Met	Lys	Ala
	180					185				190					
Leu	Met	Asn	Thr	Val	Ser	Ala	Leu	Gly	Val	Pro	Val	Gln	Gln	Asp	Phe
	195					200				205					
Leu	Cys	Gly	His	Pro	Arg	Gly	Val	Ser	Met	Ile	Met	Asn	Asn	Leu	Asp
	210					215				220					
Glu	Asn	Gln	Val	Arg	Val	Asp	Ala	Ala	Arg	Ala	Trp	Leu	Leu	Pro	Asn
	225				230				235		240				
Tyr	Gln	Arg	Ser	Asn	Leu	Glu	Ile	Leu	Thr	Gly	Gln	Met	Val	Gly	Lys
	245					250				255					
Val	Leu	Phe	Lys	Gln	Thr	Ala	Ser	Gly	Pro	Gln	Ala	Val	Gly	Val	Asn
	260					265				270					
Phe	Gly	Thr	Asn	Lys	Ala	Val	Asn	Phe	Asp	Val	Phe	Ala	Lys	His	Glu
	275					280				285					
Val	Leu	Leu	Ala	Ala	Gly	Ser	Ala	Ile	Ser	Pro	Leu	Ile	Leu	Glu	Tyr
	290				295				300						
Ser	Gly	Ile	Gly	Leu	Lys	Ser	Val	Leu	Asp	Gln	Ala	Asn	Pro	Thr	Gln
	305				310				315		320				
Leu	Leu	Asp	Leu	Pro	Val	Gly	Ile	Asn	Met	Gln	Asp	Gln	Thr	Thr	Thr
	325					330				335					
Thr	Val	Ser	Ser	Arg	Ala	Ser	Ser	Ala	Gly	Ala	Gly	Gln	Gly	Gln	Ala
	340					345				350					
Val	Phe	Phe	Ala	Asn	Pro	Thr	Glu	Thr	Phe	Gly	Asp	Tyr	Ala	Pro	Gln
	355					360				365					
Ala	Arg	Asp	Leu	Leu	Asn	Thr	Lys	Leu	Asp	Gln	Trp	Ala	Glu	Glu	Thr

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370            375            380

Val Ala Arg Gly Gly Phe His Asn Pro Thr Ala Leu Lys Val Gln Tyr  
 385                 390                 395                 400

Glu Asn Tyr Arg Asn Trp Leu Leu Asp Glu Asp Val Ala Phe Ala Glu  
 405                 410                 415

Leu Phe Met Asp Thr Glu Gly Glu Ile Asn Phe Asp Leu Trp Asp Leu  
 420                 425                 430

Ile Pro Phe Thr Arg Gly Ser Val His Ile Leu Ser Ser Asp Pro Tyr  
 435                 440                 445

Leu Trp Gln Phe Ala Asn Asp Pro Lys Phe Phe Leu Asn Glu Phe Asp  
 450                 455                 460

Leu Leu Gly Gln Ala Ala Ala Ser Lys Leu Ala Arg Asp Leu Thr Ser  
 465                 470                 475                 480

Gln Gly Ala Met Lys Glu Tyr Phe Ala Gly Glu Thr Leu Pro Gly Tyr  
 485                 490                 495

Asn Leu Val Gln Asn Pro Thr Leu Ser Gln Trp Ser Asp Tyr Val Leu  
 500                 505                 510

Gln Asn Phe Arg Pro Asn Trp His Ala Val Ser Ser Cys Ser Met Met  
 515                 520                 525

Ser Arg Glu Leu Gly Gly Val Val Asp Ala Thr Ala Lys Val Tyr Gly  
 530                 535                 540

Thr Gln Gly Leu Arg Val Ile Asp Gly Ser Ile Pro Pro Thr Gln Val  
 545                 550                 555                 560

Ser Ser His Ser Met Thr Ile Phe Tyr Gly Met Ala Leu Lys Val Ala  
 565                 570                 575

Asp Ala Ile Leu Asp Asp Tyr Ala Lys Ser Ala  
 580                 585

<210> SEQ ID NO 11  
<211> LENGTH: 33  
<212> TYPE: DNA  
<213> ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 11

ggtgttccatgtcatga ccattttcta cg 33

<210> SEQ ID NO 12  
<211> LENGTH: 33  
<212> TYPE: DNA  
<213> ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 12

ccgttagaaa tggtcatgac atggaaagac acc 33

<210> SEQ ID NO 13  
<211> LENGTH: 33  
<212> TYPE: DNA  
<213> ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 13

ggtgttccatgtcatga ccattttcta cg 33

<210> SEQ ID NO 14  
<211> LENGTH: 33  
<212> TYPE: DNA  
<213> ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 14

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ccgttagaaaa tggcatgga atggaaagac acc	33
<210> SEQ ID NO 15	
<211> LENGTH: 33	
<212> TYPE: DNA	
<213> ORGANISM: Penicillium amagasakiense	
<400> SEQUENCE: 15	
ggtgttccc cataccatga ccattttcta cg	33
<210> SEQ ID NO 16	
<211> LENGTH: 33	
<212> TYPE: DNA	
<213> ORGANISM: Penicillium amagasakiense	
<400> SEQUENCE: 16	
ccgttagaaaa tggcatggt atggaaagac acc	33
<210> SEQ ID NO 17	
<211> LENGTH: 33	
<212> TYPE: DNA	
<213> ORGANISM: Penicillium amagasakiense	
<400> SEQUENCE: 17	
ggtgttccc catattatga ccattttcta cg	33
<210> SEQ ID NO 18	
<211> LENGTH: 33	
<212> TYPE: DNA	
<213> ORGANISM: Penicillium amagasakiense	
<400> SEQUENCE: 18	
ccgttagaaaa tggcataat atggaaagac acc	33
<210> SEQ ID NO 19	
<211> LENGTH: 26	
<212> TYPE: DNA	
<213> ORGANISM: Penicillium amagasakiense	
<400> SEQUENCE: 19	
ggacaccgag ggcgagatca acttcg	26
<210> SEQ ID NO 20	
<211> LENGTH: 26	
<212> TYPE: DNA	
<213> ORGANISM: Penicillium amagasakiense	
<400> SEQUENCE: 20	
cgaagtgtat ctcgcctcg gtgtcc	26
<210> SEQ ID NO 21	
<211> LENGTH: 1769	
<212> TYPE: DNA	
<213> ORGANISM: Penicillium aculeatum	
<400> SEQUENCE: 21	
atgtacctgc ctgccaaca gattgatgtc cagtctagtc ttctcagtga ccctagcaag	60
gttgaggaa agacctatga ttacatcatt gctgggtgg gtttactgg ctttactgtt	120
gtgtccaaat tgacagaaaa ccccaagatc aaagtctgg tcattgaaaa gggcttctat	180
gagtccaaacg atggagccat catcgaggat ccaaattgtt atggacaaat ctttggcacc	240
actgttggacc agaactacct caccgttccc ctgatcaaca accgcacgaa caatatcaag	300

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ggccggtaaag gtcttggagg atcacacctg ataaacggtg actccttggac tcgcggccagac 360  
aaagtccaga ttgattcttg ggagaaggc tttggcatgg aagggtggaa ttgggacaac 420  
atgttcgagt acatgaagaa ggccgaggt gcacgtaccc ctactgctgc tcaggttgc 480  
gctggccact ccttcaatgc tacctgccat ggaaccaacg gtactgttca atccggagcc 540  
cgtgacaacg gccagccttg gtctccttatt atgaaggccc ttatgaacac cgtctcgcc 600  
cttgggtc ccgtacagca agactttc tttgggtcatac caccgggtgt ctctatgtatc 660  
atgaacaatc tcgacgaaaa ccaagttcgt gttgatgctg cccgtgcatt gctgottccc 720  
aactaccagg gctcgaattt ggagatcctt actggcaga tgggtggaaa gggttgcgtt 780  
aaacagaccc catccggtcc ccaggctgtt ggtgtgaact tcggactaa taaggccgtc 840  
aactttgacg tctttgctaa gcatgggtc cttttgggtc ctggcgtcgc tatctctccg 900  
ctgatcttgg aatattctgg cataggcttg aagtctgttc ttgataaagc caatgtcaact 960  
cagcttcttg atcttcctgt tggtatcaat atgcaagatc agaccacaac cactgtcaagt 1020  
tcccgtgcta gttccgctgg tgctggtag ggtcaggccg tcttcttcgc caatttcact 1080  
gagaccttcg gtgactacgc cccccaggcc agggacttac tcaacaccaa gctcgaccaa 1140  
tggggccgagg agaccgttgc gcgegggtgtt ttccataatg taactgctct caaagtacaa 1200  
tacgaaaact atcgtaactg gtccttgcac gaagacgtcg cttccggcga gctttcatg 1260  
gacaccggagg gccagatcaa cttegattta tgggatctca tcccttcac tcgtgggtcc 1320  
gtccatatcc tcagtagcga tccttaccta tggcaattcg ccaacgaccc caatttcact 1380  
ctgaacggat ttgacccctt tggtaagct gccgcttcca agcttgcgt tgatctcaact 1440  
agccaaggcg ctatgaagga gtacttcgac ggggagactc ttccaggata caacttggc 1500  
cagaatgcta ctcttccca tgggtcgat tatgttctac agaacttccg tcccaactgg 1560  
catgctgtga gcagctgctc tatgtatgtct agagagctt gttgggtcgt tgatgtact 1620  
gccaagggtt acggatccca aggccatcgt gtcattgcac ggtcttattcc tccgactcag 1680  
gtgttccctt attccatgac cattttctac ggaatggctt tgaagggtgc tgatggcatt 1740  
ttggatgact atgccaagg tgcctcgat 1769

<210> SEQ ID NO 22

<211> LENGTH: 588

<212> TYPE: PRT

<213> ORGANISM: *Penicillium amagasakiense*

<400> SEQUENCE: 22

Pro Ser Lys Val Ala Gly Lys Thr Tyr Asp Tyr Ile Ile Ala Gly Gly  
                  20                     25                     30

Gly Leu Thr Gly Leu Thr Val Ala Ala Lys Leu Thr Glu Asn Pro Lys  
 35                  40                  45

Ile	Lys	Val	Leu	Val	Ile	Glu	Lys	Gly	Phe	Tyr	Glu	Ser	Asn	Asp	Gly
50						55					60				

Ala	Ile	Ile	Glu	Asp	Pro	Asn	Ala	Tyr	Gly	Gln	Ile	Phe	Gly	Thr	Thr
65					70					75					80

Val Asp Gln Asn Tyr Leu Thr Val Pro Leu Ile Asn Asn Arg Thr Asn  
85 90 95

Asn Ile Lys Ala Gly Lys Gly Leu Gly Gly Ser Thr Leu Ile Asn Gly  
100 105 110

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Asp Ser Trp Thr Arg Pro Asp Lys Val Gln Ile Asp Ser Trp Glu Lys  
 115 120 125  
 Val Phe Gly Met Glu Gly Trp Asn Trp Asp Asn Met Phe Glu Tyr Met  
 130 135 140  
 Lys Lys Ala Glu Ala Ala Arg Thr Pro Thr Ala Ala Gln Leu Ala Ala  
 145 150 155 160  
 Gly His Ser Phe Asn Ala Thr Cys His Gly Thr Asn Gly Thr Val Gln  
 165 170 175  
 Ser Gly Ala Arg Asp Asn Gly Gln Pro Trp Ser Pro Ile Met Lys Ala  
 180 185 190  
 Leu Met Asn Thr Val Ser Ala Leu Gly Val Pro Val Gln Gln Asp Phe  
 195 200 205  
 Leu Cys Gly His Pro Arg Gly Val Ser Met Ile Met Asn Asn Leu Asp  
 210 215 220  
 Glu Asn Gln Val Arg Val Asp Ala Ala Arg Ala Trp Leu Leu Pro Asn  
 225 230 235 240  
 Tyr Gln Arg Ser Asn Leu Glu Ile Leu Thr Gly Gln Met Val Gly Lys  
 245 250 255  
 Val Leu Phe Lys Gln Thr Ala Ser Gly Pro Gln Ala Val Gly Val Asn  
 260 265 270  
 Phe Gly Thr Asn Lys Ala Val Asn Phe Asp Val Phe Ala Lys His Glu  
 275 280 285  
 Val Leu Leu Ala Ala Gly Ser Ala Ile Ser Pro Leu Ile Leu Glu Tyr  
 290 295 300  
 Ser Gly Ile Gly Leu Lys Ser Val Leu Asp Gln Ala Asn Val Thr Gln  
 305 310 315 320  
 Leu Leu Asp Leu Pro Val Gly Ile Asn Met Gln Asp Gln Thr Thr Thr  
 325 330 335  
 Thr Val Ser Ser Arg Ala Ser Ser Ala Gly Ala Gly Gln Gly Gln Ala  
 340 345 350  
 Val Phe Phe Ala Asn Phe Thr Glu Thr Phe Gly Asp Tyr Ala Pro Gln  
 355 360 365  
 Ala Arg Asp Leu Leu Asn Thr Lys Leu Asp Gln Trp Ala Glu Glu Thr  
 370 375 380  
 Val Ala Arg Gly Phe His Asn Val Thr Ala Leu Lys Val Gln Tyr  
 385 390 395 400  
 Glu Asn Tyr Arg Asn Trp Leu Leu Asp Glu Asp Val Ala Phe Ala Glu  
 405 410 415  
 Leu Phe Met Asp Thr Glu Gly Gln Ile Asn Phe Asp Leu Trp Asp Leu  
 420 425 430  
 Ile Pro Phe Thr Arg Gly Ser Val His Ile Leu Ser Ser Asp Pro Tyr  
 435 440 445  
 Leu Trp Gln Phe Ala Asn Asp Pro Lys Phe Phe Leu Asn Glu Phe Asp  
 450 455 460  
 Leu Leu Gly Gln Ala Ala Ala Ser Lys Leu Ala Arg Asp Leu Thr Ser  
 465 470 475 480  
 Gln Gly Ala Met Lys Glu Tyr Phe Ala Gly Glu Thr Leu Pro Gly Tyr  
 485 490 495  
 Asn Leu Val Gln Asn Ala Thr Leu Ser Gln Trp Ser Asp Tyr Val Leu  
 500 505 510  
 Gln Asn Phe Arg Pro Asn Trp His Ala Val Ser Ser Cys Ser Met Met  
 515 520 525

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Ser Arg Glu Leu Gly Gly Val Val Asp Ala Thr Ala Lys Val Tyr Gly  
530 535 540

Thr Gln Gly Leu Arg Val Ile Asp Gly Ser Ile Pro Pro Thr Gln Val  
545 550 555 560

Ser Ser His Ser Met Thr Ile Phe Tyr Gly Met Ala Leu Lys Val Ala  
565 570 575

Asp Ala Ile Leu Asp Asp Tyr Ala Lys Ser Ala Ser  
580 585

<210> SEQ ID NO 23

<211> LENGTH: 1769

<212> TYPE: DNA

<213> ORGANISM: Penicillium amagasakience

<400> SEQUENCE: 23

atgtacactgc	ctgccccaca	gattgatgtc	cagtctagtc	ttctcagtga	cccttagcaag	60
gttgcaggaa	agaccttatga	ttacatcatt	gctgggtggtg	gtttgactgg	ccttactgtt	120
gtcgccaaat	tgacagaaaa	ccccaaagatc	aaagtccctgg	tcattgaaaa	gggcttctat	180
gagtccaaacg	atggagccat	catcgaggat	ccaaatgctt	atggacaaat	cttggcacc	240
actgttgacc	agaactacct	caccgttccc	ctgatcaaca	accgcacgaa	caatatcaag	300
gccggtaaag	gtcttggagg	ataaaccttg	ataaacggtg	actcctggac	tcgcccagac	360
aaagtccaga	ttgattcttg	ggagaaggtc	tttggcatgg	aagggttgaa	ttgggacaac	420
atgttcgagt	acatgaagaa	ggccgaggct	gcacgtaccc	ctactgctgc	tcagcttgct	480
gctggccact	ccttcaatgc	tacctgccat	gaaaccaacg	gtactgttca	atccggagcc	540
cgtgacaacg	gccagccttg	gtctcctatt	atgaaggccc	ttatgaacac	cgtctcgcc	600
cttgggttcc	ccgtacagca	agactttctc	tgtggtcatc	cacgagggtgt	ctctatgatc	660
atgaacaatc	tcgacgaaaa	ccaagttcgt	gttgatgctg	cccgtgcatg	gctgcttccc	720
aactaccagc	gctcgaattt	ggagatcctt	actggtcaga	tgggtggaaa	ggttctgttt	780
aaacagaccc	catccggtcc	ccaggctgtt	ggtgtgaact	tccgtactaa	taaggccgtc	840
aactttgacg	tctttgctaa	gcatgaggtc	ctttggctg	ctggctcagc	tatctctccg	900
ctgatcttgg	aatattctgg	cataggcttg	aagtctgttc	ttgatcaagc	caatgtcaet	960
cagcttcttg	atcttcctgt	tggtatcaat	atgcaagatc	agaccacaaac	cactgtcagt	1020
tcccggtcta	gttccgctgg	tgctggtcag	ggtcaggccg	tcttcttcgc	caatttcact	1080
gagaccttcg	gtgactacgc	cccccaggcc	agggacttac	tcaacaccaa	gctcgaccaa	1140
tggcccgagg	agaccgttgc	gcgcgggtgg	ttccataatg	taactgctct	caaagtacaa	1200
tacgaaaact	atcgtaactg	gtcttttgcac	gaagacgtcg	ccttcggca	gtctttcatg	1260
gacaccgagg	gcatgatcaa	cttcgattta	tgggatctca	tccctttcac	tcgtgggtcc	1320
gtccatatacc	tcaatgcga	tcttaccta	tggcaatttcg	ccaaacgaccc	caaatttttc	1380
ctgaacacgt	ttgacactct	tggtaagct	gcccgttcca	agcttgcgtc	tgtatctact	1440
agccaaggcc	ctatgaagga	gtacttcgcc	ggggagactc	ttccaggata	caacttggtc	1500
cagaatgcta	ctctttccca	gtggtcggat	tatgtcttac	agaacttccg	tcccaactgg	1560
catgctgtga	gcagctgctc	tatgtatgtct	agagagcttg	gtgggtgtcg	tgtatgtact	1620
gccaagggtgt	acggtaacca	agggctacgt	gtcattgacg	ggtctattcc	tccgactcag	1680
gtgtcttccc	attccatgac	cattttctac	ggaatggctt	tgaagggttc	tgtatgcccatt	1740
ttggatgact	atgccaaaag	tgcctcgct				1769

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<210> SEQ\_ID NO 24  
<211> LENGTH: 588  
<212> TYPE: PRT  
<213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 24

Tyr	Leu	Pro	Ala	Gln	Gln	Ile	Asp	Val	Gln	Ser	Ser	Leu	Leu	Ser	Asp
1				5				10				15			
Pro	Ser	Lys	Val	Ala	Gly	Lys	Thr	Tyr	Asp	Tyr	Ile	Ile	Ala	Gly	Gly
	20					25					30				
Gly	Leu	Thr	Gly	Leu	Thr	Val	Ala	Ala	Lys	Leu	Thr	Glu	Asn	Pro	Lys
	35					40				45					
Ile	Lys	Val	Leu	Val	Ile	Glu	Lys	Gly	Phe	Tyr	Glu	Ser	Asn	Asp	Gly
	50				55				60						
Ala	Ile	Ile	Glu	Asp	Pro	Asn	Ala	Tyr	Gly	Gln	Ile	Phe	Gly	Thr	Thr
	65				70				75		80				
Val	Asp	Gln	Asn	Tyr	Leu	Thr	Val	Pro	Leu	Ile	Asn	Asn	Arg	Thr	Asn
	85					90				95					
Asn	Ile	Lys	Ala	Gly	Lys	Gly	Leu	Gly	Gly	Ser	Thr	Leu	Ile	Asn	Gly
	100					105				110					
Asp	Ser	Trp	Thr	Arg	Pro	Asp	Lys	Val	Gln	Ile	Asp	Ser	Trp	Glu	Lys
	115					120				125					
Val	Phe	Gly	Met	Glu	Gly	Trp	Asn	Trp	Asp	Asn	Met	Phe	Glu	Tyr	Met
	130					135				140					
Lys	Lys	Ala	Glu	Ala	Ala	Arg	Thr	Pro	Thr	Ala	Ala	Gln	Leu	Ala	Ala
	145				150				155		160				
Gly	His	Ser	Phe	Asn	Ala	Thr	Cys	His	Gly	Thr	Asn	Gly	Thr	Val	Gln
	165					170				175					
Ser	Gly	Ala	Arg	Asp	Asn	Gly	Gln	Pro	Trp	Ser	Pro	Ile	Met	Lys	Ala
	180					185				190					
Leu	Met	Asn	Thr	Val	Ser	Ala	Leu	Gly	Val	Pro	Val	Gln	Gln	Asp	Phe
	195					200				205					
Leu	Cys	Gly	His	Pro	Arg	Gly	Val	Ser	Met	Ile	Met	Asn	Asn	Leu	Asp
	210					215				220					
Glu	Asn	Gln	Val	Arg	Val	Asp	Ala	Ala	Arg	Ala	Trp	Leu	Leu	Pro	Asn
	225				230				235		240				
Tyr	Gln	Arg	Ser	Asn	Leu	Glu	Ile	Leu	Thr	Gly	Gln	Met	Val	Gly	Lys
	245					250				255					
Val	Leu	Phe	Lys	Gln	Thr	Ala	Ser	Gly	Pro	Gln	Ala	Val	Gly	Val	Asn
	260					265				270					
Phe	Gly	Thr	Asn	Lys	Ala	Val	Asn	Phe	Asp	Val	Phe	Ala	Lys	His	Glu
	275					280				285					
Val	Leu	Leu	Ala	Ala	Gly	Ser	Ala	Ile	Ser	Pro	Leu	Ile	Leu	Glu	Tyr
	290				295				300						
Ser	Gly	Ile	Gly	Leu	Lys	Ser	Val	Leu	Asp	Gln	Ala	Asn	Val	Thr	Gln
	305				310				315		320				
Leu	Leu	Asp	Leu	Pro	Val	Gly	Ile	Asn	Met	Gln	Asp	Gln	Thr	Thr	Thr
	325					330				335					
Thr	Val	Ser	Ser	Arg	Ala	Ser	Ser	Ala	Gly	Ala	Gly	Gln	Gly	Gln	Ala
	340					345				350					
Val	Phe	Phe	Ala	Asn	Phe	Thr	Glu	Thr	Phe	Gly	Asp	Tyr	Ala	Pro	Gln
	355					360				365					
Ala	Arg	Asp	Leu	Leu	Asn	Thr	Lys	Leu	Asp	Gln	Trp	Ala	Glu	Glu	Thr

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370	375	380
Val Ala Arg Gly Gly Phe His Asn Val Thr Ala Leu Lys Val Gln Tyr		
385	390	395
400		
Glu Asn Tyr Arg Asn Trp Leu Leu Asp Glu Asp Val Ala Phe Ala Glu		
405	410	415
Leu Phe Met Asp Thr Glu Gly Met Ile Asn Phe Asp Leu Trp Asp Leu		
420	425	430
Ile Pro Phe Thr Arg Gly Ser Val His Ile Leu Ser Ser Asp Pro Tyr		
435	440	445
Leu Trp Gln Phe Ala Asn Asp Pro Lys Phe Phe Leu Asn Glu Phe Asp		
450	455	460
Leu Leu Gly Gln Ala Ala Ala Ser Lys Leu Ala Arg Asp Leu Thr Ser		
465	470	475
480		
Gln Gly Ala Met Lys Glu Tyr Phe Ala Gly Glu Thr Leu Pro Gly Tyr		
485	490	495
Asn Leu Val Gln Asn Ala Thr Leu Ser Gln Trp Ser Asp Tyr Val Leu		
500	505	510
Gln Asn Phe Arg Pro Asn Trp His Ala Val Ser Ser Cys Ser Met Met		
515	520	525
Ser Arg Glu Leu Gly Gly Val Val Asp Ala Thr Ala Lys Val Tyr Gly		
530	535	540
Thr Gln Gly Leu Arg Val Ile Asp Gly Ser Ile Pro Pro Thr Gln Val		
545	550	555
560		
Ser Ser His Ser Met Thr Ile Phe Tyr Gly Met Ala Leu Lys Val Ala		
565	570	575
Asp Ala Ile Leu Asp Asp Tyr Ala Lys Ser Ala Ser		
580	585	

&lt;210&gt; SEQ ID NO 25

&lt;211&gt; LENGTH: 1769

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 25

atgtacacctgc ctgccccaca gattgatgtc cagtctagtc ttctcagtga ccctagcaag	60
gttgcaggaa agaccttatga ttacatcatt gctgggtggt gtttgactgg ccttactgtt	120
gctgccaaat tgacagaaaa ccccaagatc aaagtctgg tcattgaaaa gggcttctat	180
gagtccaaacg atggagccat catcgaggat ccaaatgctt atggacaaat ctttggcacc	240
actgttgacc agaactacct caccgttccc ctgatcaaca accgcacgaa caatatcaag	300
gccggtaaag gtcttggagg atcaccttg ataaacggtg actcctggac tcgcccagac	360
aaagtccaga ttgattcttg ggagaaggtc tttggcatgg aagggtggaa ttgggacaac	420
atgttcgagt acatgaagaa ggccgaggct gcacgtaccc ctactgctgc tcaagttgt	480
gctggccact ccttcaatgc tacctgccc ggaaccaacg gtactgttca atccggagcc	540
cgtgacaacg gccagccttgc gtctcctatt atgaaggccc ttatgaacac cgtctcgcc	600
cttgggttcc ccgtacagca agactttctc tgtggatcatc cacggaggtgt ctctatgtatc	660
atgaacaatc tcgacgaaaa ccaagttcggtt gttgatgtc cccgtgtcatg gctgtttccc	720
aactaccagg gctcgaattt ggagatcctt actggtcaga tgggtggaaa ggttctgttt	780
aaacagaccc catccggtcc ccaggctgtt ggtgtgaact tcggactaa taaggccgtc	840
aactttgacg tctttgctaa gcatgagggtc cttttggctgt ctggctcagc tatctctccg	900

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ctgatcttgg aatattctgg cataggctt g aagtctgttc ttgatcaagc caatgtcact	960
cagcttcttg atcttcctgt tggtatcaat atgcagaatgc agaccacaac cactgtcagt	1020
tcccggtcta gttcccgctgg tgctggcag ggccaggccg tcttcttcgc caatttcact	1080
gagacccctcg gtgactacgc cccccaggcc agggacttac tcaacaccaa gctcgaccaa	1140
tggggccgagg agaccgttgc gcgcgggtgt ttccataatg taactgtctt caaagtacaa	1200
tacgaaaact atcgtaactg gctccttgcac gaagacgtcg ccttcgcccga gctttcatg	1260
gacaccgagg gcttgatcaa ctgcattta tggatctca tcccttcac tcgtggtcc	1320
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cagaatgcta ctcttccca gtggtcggat tatgtcttac agaacttccg tcccaactgg	1560
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gccaagggtgt acgggtaccca aggcttacgt gtcatttgcg ggtcttattcc tccgactcag	1680
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&lt;210&gt; SEQ\_ID NO 26

&lt;211&gt; LENGTH: 588

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 26

Tyr	Leu	Pro	Ala	Gln	Gln	Ile	Asp	Val	Gln	Ser	Ser	Leu	Leu	Ser	Asp
1				5				10				15			

Pro	Ser	Lys	Val	Ala	Gly	Lys	Thr	Tyr	Asp	Tyr	Ile	Ile	Ala	Gly	Gly
		20					25					30			

Gly	Leu	Thr	Gly	Leu	Thr	Val	Ala	Ala	Lys	Leu	Thr	Glu	Asn	Pro	Lys
	35					40					45				

Ile	Lys	Val	Leu	Val	Ile	Glu	Lys	Gly	Phe	Tyr	Glu	Ser	Asn	Asp	Gly
	50				55				60						

Ala	Ile	Ile	Glu	Asp	Pro	Asn	Ala	Tyr	Gly	Gln	Ile	Phe	Gly	Thr	Thr
	65				70				75			80			

Val	Asp	Gln	Asn	Tyr	Leu	Thr	Val	Pro	Leu	Ile	Asn	Asn	Arg	Thr	Asn
		85					90				95				

Asn	Ile	Lys	Ala	Gly	Lys	Gly	Leu	Gly	Gly	Ser	Thr	Leu	Ile	Asn	Gly
	100						105				110				

Asp	Ser	Trp	Thr	Arg	Pro	Asp	Lys	Val	Gln	Ile	Asp	Ser	Trp	Glu	Lys
	115					120				125					

Val	Phe	Gly	Met	Glu	Gly	Trp	Asn	Trp	Asp	Asn	Phe	Glu	Tyr	Met	
	130				135				140						

Lys	Lys	Ala	Glu	Ala	Ala	Arg	Thr	Pro	Thr	Ala	Ala	Gln	Leu	Ala	Ala
	145				150				155			160			

Gly	His	Ser	Phe	Asn	Ala	Thr	Cys	His	Gly	Thr	Asn	Gly	Thr	Val	Gln
	165					170			175						

Ser	Gly	Ala	Arg	Asp	Asn	Gly	Gln	Pro	Trp	Ser	Pro	Ile	Met	Lys	Ala
	180					185					190				

Leu	Met	Asn	Thr	Val	Ser	Ala	Leu	Gly	Val	Pro	Val	Gln	Gln	Asp	Phe
	195					200				205					

Leu	Cys	Gly	His	Pro	Arg	Gly	Val	Ser	Met	Ile	Met	Asn	Asn	Leu	Asp
	210				215				220						

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Glu Asn Gln Val Arg Val Asp Ala Ala Arg Ala Trp Leu Leu Pro Asn  
 225 230 235 240  
 Tyr Gln Arg Ser Asn Leu Glu Ile Leu Thr Gly Gln Met Val Gly Lys  
 245 250 255  
 Val Leu Phe Lys Gln Thr Ala Ser Gly Pro Gln Ala Val Gly Val Asn  
 260 265 270  
 Phe Gly Thr Asn Lys Ala Val Asn Phe Asp Val Phe Ala Lys His Glu  
 275 280 285  
 Val Leu Leu Ala Ala Gly Ser Ala Ile Ser Pro Leu Ile Leu Glu Tyr  
 290 295 300  
 Ser Gly Ile Gly Leu Lys Ser Val Leu Asp Gln Ala Asn Val Thr Gln  
 305 310 315 320  
 Leu Leu Asp Leu Pro Val Gly Ile Asn Met Gln Asp Gln Thr Thr Thr  
 325 330 335  
 Thr Val Ser Ser Arg Ala Ser Ser Ala Gly Ala Gly Gln Gly Gln Ala  
 340 345 350  
 Val Phe Phe Ala Asn Phe Thr Glu Thr Phe Gly Asp Tyr Ala Pro Gln  
 355 360 365  
 Ala Arg Asp Leu Leu Asn Thr Lys Leu Asp Gln Trp Ala Glu Glu Thr  
 370 375 380  
 Val Ala Arg Gly Gly Phe His Asn Val Thr Ala Leu Lys Val Gln Tyr  
 385 390 395 400  
 Glu Asn Tyr Arg Asn Trp Leu Leu Asp Glu Asp Val Ala Phe Ala Glu  
 405 410 415  
 Leu Phe Met Asp Thr Glu Gly Leu Ile Asn Phe Asp Leu Trp Asp Leu  
 420 425 430  
 Ile Pro Phe Thr Arg Gly Ser Val His Ile Leu Ser Ser Asp Pro Tyr  
 435 440 445  
 Leu Trp Gln Phe Ala Asn Asp Pro Lys Phe Phe Leu Asn Glu Phe Asp  
 450 455 460  
 Leu Leu Gly Gln Ala Ala Ala Ser Lys Leu Ala Arg Asp Leu Thr Ser  
 465 470 475 480  
 Gln Gly Ala Met Lys Glu Tyr Phe Ala Gly Glu Thr Leu Pro Gly Tyr  
 485 490 495  
 Asn Leu Val Gln Asn Ala Thr Leu Ser Gln Trp Ser Asp Tyr Val Leu  
 500 505 510  
 Gln Asn Phe Arg Pro Asn Trp His Ala Val Ser Ser Cys Ser Met Met  
 515 520 525  
 Ser Arg Glu Leu Gly Gly Val Val Asp Ala Thr Ala Lys Val Tyr Gly  
 530 535 540  
 Thr Gln Gly Leu Arg Val Ile Asp Gly Ser Ile Pro Pro Thr Gln Val  
 545 550 555 560  
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 565 570 575  
 Asp Ala Ile Leu Asp Asp Tyr Ala Lys Ser Ala Ser  
 580 585

&lt;210&gt; SEQ ID NO 27

&lt;211&gt; LENGTH: 33

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Penicillium amagasakiense

&lt;400&gt; SEQUENCE: 27

ggacaccgag ggccagatca acttcgattt atg

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<210> SEQ ID NO 28
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 28
cataaatcga agttgatctg gccctcggtg tcc 33

<210> SEQ ID NO 29
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 29
ggacaccgag ggcatgtca acttcgattt atg 33

<210> SEQ ID NO 30
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 30
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<210> SEQ ID NO 31
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 31
ggacaccgag ggcttgatca acttcgattt atg 33

<210> SEQ ID NO 32
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Penicillium amagasakiense

<400> SEQUENCE: 32
cataaatcga agttgatcaa gccctcggtg tcc 33

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The invention claimed is:

1. A glucose oxidase (GO<sub>x</sub>) mutant with a percentage of identity of at least 95%, relative to the wild-type GOx of *Penicillium amagasakiense*, characterized in that its amino acid in position 564, with reference to the protein sequence of the wild-type GOx of *Penicillium amagasakiense* of SEQ. ID. No. 2, is replaced with an amino acid selected from the group consisting of a serine (V564S mutant), a threonine (V564T mutant) or an isoleucine (V564I mutant).

2. The GOx mutant as claimed in claim 1, characterized in that the V564S mutant also comprises a replacement of the lysine in position 424 with a glutamic acid (V564S+K424E mutant), glutamine (V564S+K424Q mutant), methionine (V564S+K424M mutant) or leucine (V564S+K424L mutant).

3. The GOx mutant as claimed in claim 1, characterized in that it has an amino acid sequence selected from the group consisting of SEQ. ID. No. 4, 6, 8, 10, 22, 24 and 26.

4. An isolated nucleic acid molecule, characterized in that it codes for a GOx mutant as claimed in claim 1.

5. The nucleic acid molecule as claimed in claim 4, characterized in that it is obtained by mutation of the nucleic acid

45 molecule of sequence SEQ. ID. No. 1 with an oligonucleotide pair selected from the group consisting of pairs of SEQ. ID. No. 13 and 14; 15 and 16; 17 and 18; 19 and 20; 27 and 28; 29 and 30 and 31 and 32.

50 6. The nucleic acid molecule as claimed in claim 4, characterized in that it has a sequence selected from the group consisting of SEQ. ID. No. 3, 5, 7, 9, 21, 23 and 25.

7. An expression vector, characterized in that it comprises a nucleic acid molecule as claimed in claim 4.

8. An isolated host cell expressing an enzyme, characterized in that it is transformed with an expression vector as claimed in claim 7.

55 9. The method of use of a GOx mutant as claimed in claim 1, for measuring the glucose concentration in a sample.

10. The method as claimed in claim 9, characterized in that the sample is a biological sample, and in particular is blood.

60 11. A glucose assay kit, characterized in that it comprises a GOx mutant as claimed in claim 1.

12. A glucose electrode, characterized in that it comprises a conductive material covered with a deposit comprising at least one GOx mutant as claimed in claim 1.

65 13. A glucose sensor, characterized in that it consists of an electrode as claimed in claim 12.

**14.** A glucose biocell, characterized in that it comprises a first electrode as claimed in claim **12** as anode and a second electrode as cathode.

**15.** A process for assaying in solution glucose of a sample, characterized in that it comprises the following steps:

- a) introduction into said sample of a redox reagent whose reduction leads to a color change and of a GOx mutant as claimed in claim **1**;
- b) measurement of the coloration intensity of the sample after enzymatic reaction;
- c) comparison of the coloration intensity measured in step b) with the intensity measured for standard solutions having a known glucose content;
- d) determination of the glucose concentration of said sample.

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**16.** A process for assaying the glucose of a sample, characterized in that it comprises the following steps:

- a) introduction into said sample of a glucose electrode as claimed in claim **12**;
- b) measurement of the intensity of the current in the sample;
- c) comparison of the intensity of the current measured in step b) with the intensity measured for standard solutions having a known glucose content;
- d) determination of the glucose concentration of said sample.

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